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Epididymal structure and function: a historical and critical review

by

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1. Introduction

In their review of the secretory functions of male accessory organs of reproduction in mammals, MANN and LUTWAK-MANN (1951) remarked that with the exception of work on prostatic secretions of man and dog, investigations on the secretions produced by individual accessory sex organs are few, and analytical studies are often limited to the seminal plasma as a whole. It is true that studies of the total male ejaculate have played an impressive rôle in elucidating problems of reproductive physiology in Man and animals, but the functional importance of the various accessory sex organs which constitute it still awaits appraisal. The epididymis in particular has received scant attention from this point of view, and the purpose of this article is to review what is known concerning epididymal structure and function and to indicate where a correlation of structure and function might be permissible.

According to one view, two functional types of testis can be distinguished in the vertebrates: those in which spermiogenesis is followed by maturation and storage of the spermatozoa within the testis (e.g. Frog, *Gasterosteus aculeatus*, etc.), and those in which the spermatozoa reach full maturity elsewhere in the male reproductive tract (e.g. Rabbit and many mammals). It follows that there are two different kinds of vertebrate epididymis: one type acting as a simple

duct and another functioning as a complex organ adapted to the ripening and storage of spermatozoa, (NEILSON, 1953). But although it has been believed for many years that the spermatozoa in most mammals undergo a 'ripening' or maturation process after they leave the testicle, it has never been clearly established whether or not the factors governing such 'ripening' are intrinsic to the spermatozoa or reside in the epithelium that surrounds them. This problem is obviously fundamental in epididymal physiology; for if the spermatozoa leave the testicle completely equiped for 'ripening', the epididymal canal, no matter how complicated it may be structurally, must play only a passive part as an excretory duct. And if the spermatozoa are not so equiped, then the epididymis may have a more dynamic contribution to make in reproductive physiology.

Thus two aspects of the problem have to be examined: whether the spermatozoa do, in fact, ripen as they descend the epididymis, and whether the epididymal epithelium is involved in the process. 'Ripening', here, is taken to mean functional maturation. It has been studied by investigating qualitative and quantitative changes in the spermatozoa as they descend the epididymis, such as the development of their intrinsic motility and fertilizing power, their morphology and metabolism.

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2. The ripening of the spermatozoa

(a) MOTILITY

TOURNADE (1913), studied the motility of the spermatozoa of the cat, guinea-pig, rat and rabbit and found that the sperm obtained from the testicular seminiferous tubules were actively motile. Those from the *cauda epididymis* exhibited progressive movements, and the *capital* sperm were only feebly active. But although he admitted the possibility of autonomous evolution of motility in spermatozoa, Tournade thought it more likely that the epididymal epithelium had an 'excito-motrice' effect upon them. In this he was supported by BENOIT (1926), and by MOORE (1928), who reported weak vibratile movements in testicular spermatozoa in the rat (but not in the guinea-pig) when the cells were suspended in normal physiological saline, and by COLLERY (1943), and REDENZ (1924). The differences of motility shown by spermatozoa at different epididymal levels of sheep, goat and buffalo are discussed by MUKHERJEE and BHATTACHARAYA (1949). KIRILOV and MOROSOV (1936) demonstrated the efficiency of the surgically-isolated bull epididymis in preserving the activity of the spermatozoa for at least 37 days.

On the other hand, YOUNG (1929, 1931) and TOOTHILL and YOUNG (1931) stated that the spermatozoa do not show progressive movements while passing through the epididymis; and LARDY and PHILLIPS (1945), studying the metabo-

lism of bovine epididymal semen, observed that sperm samples taken directly from the *cauda epididymis* were at first almost completely immobile. Within a few minutes however motility developed rapidly and was favourably influenced by aeration and agitation of the specimen.

Clearly there has been no unanimity in the past as to what constitutes motility in spermatozoa and there have been many arbitrary distinctions between what has been called *motilitas latenta* and *motilitas manifesta*. The vigorous progressive movement of normal spermatozoa seems to be a unique phenomenon seen only in freshly ejaculated semen. Nevertheless, under some circumstances oscillatory movements of the tail can be elicited in testicular sperm in some species (e.g. rat and Man), and this propensity seems to develop as the cells move towards the *cauda epididymis*. In this sense, 'ripening' may be said to have occurred.

(b) FERTILIZING POWER

The assumption that the power of movement in spermatozoa is a reliable index of fertilizing ability was for long implied in the early studies of mammalian semen (*vide* LLOYD-JONES and HAYES, 1918). But, DETLEFSON (1905), studying the motility of spermatozoa in hybrid guinea-pigs, found that 10.2 % of his animals were sterile, in spite of the fact that actively motile sperm could be obtained by incising the *cauda epididymis*; and LILLIE (1915) demonstrated that *Arbacia* sperm retain their capacity for motility long after their fertilizing power is lost. This was confirmed as true for the rabbit by HAMMOND and ASDELL (1926).

The time normally taken by the spermatozoa to traverse the epididymis from *caput* to *cauda* is uncertain. VON LANZ (1930), quoting EXNER (1904) and LODE (1891), suggested that two days are required for the journey in Man and dog. TOOTHILL and YOUNG (1931) injected suspensions of India ink into the *caput epididymis* of the guinea-pig and followed the course of the ink particles. Their results showed that 14 to 19 days were necessary for the ink to reach the *cauda epididymis* in sexually mature males; and in a group of animals in which the epididymis was isolated from the testis by ligaturing the *ductuli efferentes*, 25 to 28 days were required. Apparently avian species differ from mammalian in this respect. ALVERDES (1924) investigating the epididymis of the sparrow, and MUNRO (1938) working on the domestic fowl, agreed that the spermatozoa pass very quickly through the epididymis (twenty four hours in the cock).

KOZELUHA (1941), studied the refilling time of the epididymis of the rabbit after frequently-repeated electrically-stimulated ejaculations. He observed that it took twelve to fourteen ejaculations repeated at intervals as short as five minutes to empty the *cauda epididymis*, which was completely refilled twenty-four hours after the last ejaculation.

LLOYD-JONES and HAYES (1918) investigated the effects of excessive sexual activity on male rabbits, and noted that the vitality of the spermatozoa decreases as the number of ejaculations increases. Similar results were reported for Man (MANTEGAZZA, 1866), horse (LEWIS, 1911), dog (AMANTEA and KRYSZKOWSKI, 1921; KRYSZKOWSKI and PAWLOW, 1927); and bulls (LAGERLOF, 1936). This means that when ejaculations are repeated at short intervals 'unripe' spermatozoa appear in the *cauda epididymis*. KOZELUHA (1941) was inclined to believe that the vitality of the sperm is dependent upon the physicochemical state of the secretions produced by the glands of the reproductive system.

The length of the epididymal canal in mammals has perhaps not been sufficiently appreciated, and the figures quoted in the literature may occasion surprise. In the horse GHETIE (1939) has reported a length of 84 to 86 meters, and the following figures obtain in other species:—bullock, 40 to 50 (HARCENKO, 1930); sheep, 50 to 58 (GAVRILLA, 1932); pig, 62 to 64 (GHETIE, 1939); dog 5 to 8 (MUNTEANU, 1930); cat, 1.5 to 3 (BUSILA, 1930); and hare 2.5 to 3 (ST. GALLIS, 1933). The length of the epididymis in man is variously given in text books as 3 metres and 6 to 7 metres (TESTUT, 1901).

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(c) MORPHOLOGY AND METABOLISM

The existence of a 'middle piece bead' (MPB) in the ejaculated spermatozoa of most placental mammals has been known for some time; but it is only within comparatively recent years that its origin has been associated with the epididymis. GATENBY and COLLERY (1943) remark that RETZIUS (1909) showed an MPB in the majority of his diagrams of mammalian spermatozoa. This structure was again noted by WILLIAMS (1932), and by LAGERLOF (1938), who assumed that it persists as a cytoplasmic remainder of the spermatid stage which is extruded when the cell becomes a mature spermatozoon; but no definite function appears to have been attributd to it apart from numerous vague suggestions that in sperm cultures, motility is lost earlier by those sperm having no 'bead'. POPA and MARZA (1931) stated, without adducing proof, that the MPB is the locomotor centre of the sperm. MUKHERJEE and BHATACHARAYA (1949), described the position of the MPB in sperm from various levels of the reproductive tract in the sheep, goat, and buffalo, and give the literature concerning the MPB in the pig, ram, dog, rabbit, rat, guinea-pig and golden hamster.

COLLERY (1943) removed sperm from various levels of the dog epididymis by means of a fine pipette, and observed that all sperm within the *caput epididymis* have a MPB next to the head of the cell, and that sperm within *cauda epididymis* have a similar bead attached to the tail. Only within a small zone at the base of the *caput* and the beginning of the *corpus epididymis* is the MPB actually in the middle of the 'middle piece'. It seemed clear to this author that rearward migration of the MPB occurs and that it happens rapidly. In the

cauda epididymis he found sperm with anteriorly placed beads only in rare instances. LAGERLOF (1936) maintained that in bulls this type of spermatozoon is more often seen in animals with reduced fertility.

Apart from a suggestion by METALNIKOV (1911) that the epididymal secretions were protective to the spermatozoa, shielding them from the harmful effects of certain toxins and alkaloids, and STIGLER'S (1918) description of what he regarded as an acquired heat tolerance in sperm as they descended the epididymis, few metabolic studies of note appear until recent years.

That epididymal sperm possess metabolic characteristics far different from those of ejaculated sperm was reported by HENLE and ZITTLE (1942), who clearly demonstrated that washed and unwashed epididymal spermatozoa fail to exhibit a constant respiratory rate. They found a substance in the epididymal secretions which stabilized sperm respiration. Further, they encountered a lower endogenous respiration in the epididymal than in the ejaculated sperm; glucose had a stimulating effect on the respiration of epididymal, but depressed the endogenous respiration of ejaculated sperm. The metabolic differences between epididymal and ejaculated bovine sperm have been reviewed by LARDY, GHOSH and PLAUT (1949), and more recently, GHOSH and LARDY (1952) discovered a yeast-stimulating agent in mammalian spermatozoa which seems to occur in an inactive form in epididymal sperm.

The facts assembled in the foregoing section indicate that spermatozoa do indeed 'ripen' as they descend the epididymis. They do not clarify whether the sperm 'ripen' by reacting to stimuli emanating from the environmental epithelium or whether they simply mature as time passes, environment being indifferent in that respect.

3. Regional structure

As described in the preceeding section, sperm from different regions of the epididymis possess differing biological properties. In this section, the general structure of the canal will be described and the regional differences will be emphasized.

(a) MICROANATOMICAL

There are gross regional microanatomical and cytological differences along the epididymis. BENOIT (1926) distinguished two main groups of animals on the basis of the relationship between the *caput epididymis* and the testicular pole. There are those in which the *caput* is composed of conical lobules (the *coni vasculosi* of the anatomists) found, for example in man, dog, cat, bull, horse and ram; and those in which the *caput* is formed entirely by the *ductus*

epididymis into which the *ductuli efferentes* open independently or by a single terminal duct, for example, in the rabbit, guinea-pig, mouse and rat. In the latter group, the epididymis is not intimately attached to the testicular pole, and the short transitional zone between the terminal *ductus deferens* and the initial segment of the epididymis shows striking changes in the epithelium. The typical low columnar epithelial cells of the ductuli are replaced by tall cells of the initial segment in a patchy manner. The patches of tall cells become more frequent until, in the initial segment proper, they alone remain.

The tubule forming the initial segment is tightly coiled and the microanatomical pattern of transverse histological sections is that of a packed group of regularly disposed sections of the tube. Another feature of the region is that it is often empty of spermatozoa. HAMMAR (1897) suggested that the initial segment might be a *cul-de-sac,* thus accounting for the paucity of its contents; but BENOIT'S view (1926) is that the activity of the motile cilia of the *ductuli efferentes* drives the sperm rapidly through the initial segment, so that sections of the region rarely show many spermatozoa in the lumen of the tube.

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An interesting feature of the epididymis in most mammals is the gradual increase of tubule diameter from the cranial to the caudal end, and *pari passu* with this, a decrease in the height of the columnar epithelial cells which line its lumen. In man, for instance, the height of the epithelial lining of the initial segment varies from 55—65 μ, whereas the measurements for those of the *corpus* and *cauda* are given as 25—30 μ and 20—30 μ respectively (BENOIT, 1926). The stereocilia (*vide* Section 5), also decrease in height from the *caput* towards the *cauda.*

On this evidence alone it might be expected that these changes in epithelial morphology might reflect differences in the physiology and secretory activity of the cells in the *caput* and *cauda epididymis,* and this is borne out by cytological studies. In the rat, for example, it has been reported (MANEELY, 1954) that there is strong evidence of a secretory cycle throughout the epididymis, but that its rhythm is probably slower in the *cauda* than in the *caput* (in the lower portion of which it may be maximal). Moreover, recent histochemical studies indicate that there are differences in the alkaline phosphatase activity of the *caput* and *cauda epididymis (vide infra).*

The musculature of the different parts of the mammalian epididymis has been the subject of much controversy. Several authors have denied its presence except in the caudal portion of the tail, e.g. HAMMAR (1897), working on dogs. But AIGNER (1900) and SPANGARO (1902), working on human material, HENRY (1900) on the rabbit, rat, guinea-pig and dog, and BENOIT (1926) on the rat, found smooth muscle around the tube wall throughout the epididymis. Obviously the distribution of smooth muscle in the epididymis will have some bearing on the efficiency of ejaculation and on the refilling time of the *cauda* after it has been emptied of spermatozoa; but, apart from KOZELUHA'S (1941) experiments

previously noted, insufficient work has been done on the different animal species to justify conjecture as to the physiological significance of the mammalian epididymal musculature. It should be noted that there are excellent recent accounts of epididymal differentiation in birds by MARAUD (1955) and of the structure of the genital apparatus (including the epididymis) of the field mouse *(Microtus arvalis)* by DELOST (1956).

In view of these regional structural differences in the epididymis, it is of some importance whether similar regional histochemical differences can be demonstrated which might point to a functional differentiation of its various parts. Within recent years, histochemical techniques have been devised which are thought to demonstrate, in visible form, the presence of organic compounds known to be intimately connected with physiological functions in cells and tissues. Such are the periodic acid-Schiff reaction for the demonstration of polysaccharide complexes, and the GOMORI-TAKAMATSU technique (1939) for the demonstration of alkaline phosphatase.

(b) HISTOCHEMICAL

(1) *Polysaccharide complexes*

LEBLOND (1950) gave one illustration of the distribution of periodic acid-Schiff (PAS) positive material in the *caput epididymis* of the rat (the exact portion of the *caput* unspecified), and stated that the epithelium showed a weak staining reaction in the cytoplasm and in the stereocilia. The Golgi zone showed deeply staining granules associated with an outer unstained framework. Unstained vacuoles occupied the cells. MONTAGNA and HAMILTON (1952) found glycogen in the basal cells of the human epididymis, and PAS-positive diastase-resistant granules were seen in the other cellular components of the epithelium. They confirmed Leblond's observation concerning the Golgi zone. More recently, NICANDER (1954) has demonstrated glycogen in the epididymal epithelium of the rabbit and dog, and stated that he has other evidence of specific differences in the amount of glycogen demonstrable. In a contribution to the histochemistry of the epididymis, MANEELY (1955) reported the distribution of PAS-positive material in the various parts of the rat epididymis of which the following were the principal observations.

Throughout the epididymis the basement membrane, the collagen fibres of the *membrana propria,* and the endothelial lining of the vascular system were all PAS-positive. The epithelial cells, on the other hand, showed marked differences in the various anatomical regions of the organ. The tall columnar epithelial cells of the initial segment of the *caput* showed a few intra-cytoplasmic PAS-positive granules, and the numerous small cytoplasmic vacuoles present were PAS-negative, apart from a weak positive reaction on their rims. The cytoplasm

itself showed a weak diffuse positive reaction, and the stereocilia were strongly positive.

In the middle portion of the *caput* an increase in the number of PAS-positive intra-cytoplasmic granules was obvious in the Golgi zone, but they were rather diffusely dispersed and few were seen in the basal parts of the cells. The stereocilia were again positive.

The lower portion of the *caput* contrasted markedly with the upper portions both in the intensity of the reaction and in the distribution of the secretion granules and vacuoles. In all the animals examined the Golgi zone showed clumps of strongly positive granules, as well as many vacuoles containing positive granular material and having PAS-positive rims. Many cells showed a perinuclear distribution of secretory material and all of them carried positively reacting stereocilla.

In the *cauda epididymis,* the cells of the upper portion showed a diffuse positive cytoplasmic reaction and few PAS-positive granules were present. The middle and lower caudal regions, however, showed an increase both in secretory activity, as judged by the number and distribution of granules within the cells, and the strength of the PAS reaction. About one in twenty cells were packed with PAS-positive granules and are probably identical with the large cells with 'foamy' cytoplasm seen in ordinary histological sections of the *cauda epididymis* in the rat. In spite of this, the author had the distinct impression that whilst a secretory cycle is present throughout the epididymis, its rhythm is slower in the *cauda* than in the lower portion of the *caput,* where it appears to be maximal. These observations were, of course, purely subjectively controlled; but must stand at present in view of the difficulty of measuring exactly the quantity of PAS-positive material in the cells, or of identifying precisely its chemical constitution, by histochemical methods.

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(2) *Alkaline phosphatase*

Regarding the distribution of alkaline glycerophosphatase, DEAN and DEMPSEY (1945) state that the enzyme is demonstrable only on the surface of the epithelial cells in the rabbit and the rat. SOULAIRAC and THIBUALT (1946) described the localization of the enzyme in the rat genital tract before and after castration. They found that it was absent from the epithelium and that while the *caput* and the *corpus* are lacking in alkaline phosphatase activity, the *membrana propria* of the caudal tubules was positive. WISLOCKI (1949) reported seasonal changes in the distribution of alkaline phosphatase in the testes, seminal vesicles and epididymides of deer, the stereocilia and the basal cells being strongly positive only in the autumn, the basement membrane reacting moderately. ZORZOLI and STOWELL (1947) compared the distribution of hexose-diphosphatase with glycerophosphatase in different tissues and stated that, in the epi-

TABLE I. Summary of the distribution of alkaline glycerophosphatase in the rat epididymis.

Incubation time (min.)	Caput		Cauda		Intra-epididymal portion of Ductus Deferens	
	BM	BVV	BM	BVV	BM	M
½	0	1	0	1	0	1
3	1	4	1	4	1	4
5	2	4	1	4	2	4
10	3	5	2	5	3	4
15	5	5	2	5	4	4
30	5 Y	5	3	5	5	5 X
60	5 Y	5	4 X	5	5	5 Y

BM = basement membrane
M = peritubular smooth muscle
X = evidence of diffusion
Y = marked diffusion
BVV = endothelium of certain blood vessels

0 = no reaction
1 = faint patchy reaction
2 = patchy reaction
3 = complete impregnation of BM
4 = strong reaction
5 = very strong reaction

didymis, glycerophosphatase is present in the nuclei of the sub-epithelial connective tissue. BERN (1949) produced the first comparative study of the distribution of alkaline phosphatase in the genital tract of mammals and reported that it was found in the epithelium of the *caput epididymis* (the stereocilia showing a particularly strong reaction), whereas the caudal epithelium was negative. MANEELY (1955) reported the distribution of alkaline glycerophosphatase in the rat epididymis; the principal observations being summarized in Table 1. It will be noted that although the presence of alkaline phosphatase has been established in the epididymis, there are different opinions as to its precise distribution. It is difficult to find a basis for comparison of the results reported in the literature since in some instances the materials and methods used in the experiments and of the controls and incubation times employed are lacking. On the other hand it is obvious from other reports in the literature that excessive periods of incubation were used, which, in the light of recent experience almost certainly must have produced false localizations of the enzyme. Moreover, the photographs in many papers on alkaline phosphatase histochemistry show nuclear reactions varying from a distinct darkening of the nuclear membrane to intense blackening of the whole nuclear structure. NOVIKOFF (1952) states that 'Rigid biochemical proof for the presence of alkaline phosphatase in nuclei has yet to be produced'. He does not claim that nuclei do not possess alkaline phosphatase, and admits that there could be differences among nuclei of the various organs. He does claim, however, that no evidence of its presence can be obtained from the staining or degree of staining of nuclei with the Gomori-Takamatsu technique, and states: 'If alkaline phosphatase is present in nuclei, it is of low level of

magnitude—too low to be demonstrated histochemically!' This view is generally accepted at the present time. The author's observations on the distribution of alkaline phosphatase (Table 1) were based on three assumptions. Firstly that a marked nuclear reaction, irrespective of the incubation time, indicated a false positive result produced by diffusion of the enzyme or its reaction products with subsequent adsorption on nuclei (YOKOYAMA, STOWELL and MATHEWS, (1951); LEDUC and DEMPSEY, (1951); NOVIKOFF, (1952); and MOOG, (1951). Secondly, that the extent of blackening of sections does not necessarily reflect the degree of enzyme activity. Thirdly, that the point on the time scale at which a positive reaction in sections incubated for serial periods occurs may have much more significance than the intensity of the blackening. The author was unable to demonstrate alkaline phosphatase in the nuclei of the epithelial cells in the epididymis, or in their organelles or stereocilia, with brief periods of incubation. But the results showed that there was a higher level of activity of the enzyme in the basement membrane and *membrana propria* in the *caput epididymis* than in the *cauda* (excluding the intraepididymal portion of the *ductus deferens*).

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Whilst the role of alkaline phosphatase in tissues is conjectural, one view (BRADFIELD, (1950); MOOG and WENGER, (1952) is that the enzyme is involved in the transport of organic molecules inward, outward, and across cells. This view was partly founded on the fact that the enzyme is demonstrable in locations ideally suited for participation in such dynamic processes. MOOG and WENGER (1952) suggested that there is a correlation between mucopolysaccharides and alkaline phosphatase and have shown that at the points of maximum alkaline phosphatase activity in certain tissues, such as the striated border of the duodenal epithelium, mucopolysaccharides are also present. It is interesting to note that a similar association has come to light in the epididymis (MANEELY, 1955, 1958).

4. Cytological evidence of secretion

Much has been written concerning the evidence that the epididymal epithelium secretes substances into the lumen of the tube. The first contribution of importance was that of VAN DER STRICHT (1893) who noticed 'safranophilic' granules in the epithelial cells in Lizards. These granules were surrounded by a clear hyaline zone; the surface of the cells carried clear hyaline vesicles, and 'safranophilic' granules could be found among the sperm mass in the centre of the tube. He quoted VON EBNER'S opinion (1888) that the epididymal epithelium nourishes the sperm. SCHAFFER (1892) described secretory appearances in the human epididymis and drew similar conclusions.

In 1897, Hammar described secretion granules in the dog epididymis, the secretory phenomena apparently occurring in 4 phases:— (1) Rest: the cells

having an 'indifferent' character. (2) Activity: the 'spongioplasm' becomes filled with granules. (3) Secretion: the cells loose their cilia and empty their globules into the lumen. (4) Reconstruction of the cells. An interesting feature of Hammar's observations is that he believed the nuclei took part in the formation of secretion. This observation recurs in LUDFORD'S (1925) studies *(vide infra)*.

The most extensive investigation of the epididymis at this period was that of HENRY (1900), whose material included seven reptilian species, two avian (dove, domestic fowl) and four mammalian (dog, rabbit, rat, and guinea-pig). Henry considered secretion as occuring in three phases, as follows. First, nuclear secretion; second, nuclear excretion and cytoplasmic phase; and third, cytoplasmic excretion. Secretion products were able to leave the cell in reptiles, birds and mammals as a result, first, of an increase of intracellular pressure due to accumulation of secretion globules; the rupture of the cell surface; and the escape of the secretion globules carrying a quantity of cytoplasm with them, followed by a loss in the cells' height. Henry's experimental work on the epididymis consisted of ligature and resection of the *vas deferens* in the rat. The animals were examined after 10, 19 and 32 days; the secretory phenomena had ceased 10 days after the operation and the epididymis was remarkably reduced in volume after 19 days. He reported signs of 'degeneration' of the epithelium in all his specimens and attributed this to the regression which occured in the testicle after the operation. A healthy functioning testicle was, it seemed, necessary for the maintenance of secretion in the epididymis.

REGAUD (1901) was the first to describe the compound nature of secretion droplets in the epididymis. Using a fixative containing potassium bichromate and acetic acid, he stained his specimens with haematoxylin, after mordanting with copper salts and decolourizing in borax ferricyanide. The outer zone of the secretion droplets stained darkly, whilst their centres remained unstained. Regaud believed that his secretion droplets and those of Henry represented two different entities, Henry's being much less abundant and having a different morphology. It should be borne in mind however that Regaud's technique was subjectively controlled.

COURRIER (1920) made an interesting contribution to the problem of epididymal secretion and its probable necessity for the maintenance of sperm vitality. In animals such as the lizard showing continuous spermatogenic activity, the cells of the *ductuli efferentes* and *caput epididymis* always show secretory activity (see also: VAN DER STRICHT, 1893, and HENRY, 1900) but in those species in which spermatogenesis is seasonal or periodic, continuous secretory activity is not seen. In the bat, for instance, on account of its winter hibernation, the importance of the epididymal secretion is well shown since during hibernation and after the cessation of spermatogenesis, the tail of the epididymis is packed with spermatozoa and a secretion produced in the initial segment. Thus the di-

stal segment has become a reservoir whilst the proximal part is differeniated as a glandular organ.

Courrier described different stages in secretory activity in the inital segment and pointed out that the sperm remain and live within a duct where, he affirmed, they are denied access to nutritive material. The presence of 'alimentary' substances elaborated by their storehouse is thus rendered necessary, and the secretions produced by the head of the epididymis must fill this rôle. Further evidence in favour of this was that large secretory granules could be seen in the epididymal canal, around which the spermatozoa orientated themselves as if drawn by a 'trophotactisme electif' recalling the embedding of newly-formed spermatozoa in the cytoplasm of the Sertoli cells. Courrier stated that the sperm cells could be seen arranging themselves in bundles orientated perpendicularly to the epithelium and directing their heads towards the cytoplasm packed with secretory material. He averred that during hibernation in the bat the interstitial cells of the testis are well marked. But this is contrary to the statement of Hanseman and Ganfini (quoted by COURRIER, 1920) that in hibernators (in their experiments the marmot and hedgehog) the interstitial gland is at rest during hibernation. WISLOCKI (1949) initiated interesting histochemical studies on the seasonal changes in the epididymis of deer: he found little or no alkaline phosphatase in the epididymal epithelium when spermatogenesis is in abeyance; but during Autumn there is an intense reaction, particularly in the *stereocilia* and in the cytoplasm of the basal epithelial cells. These results, however, await confirmation and the work should be extended to include different hibernating species.

According to BENOIT (1926), at least two kinds of secretion are produced by the epididymal epithelial cells: a liquid product, formed close to the Golgi apparatus, appearing in the form of vacuoles which rise slowly towards the apical cytoplasm: and granules, originating in the same region. In some species (especially in the bull) 'osmophilic' substances can be found, and in the rat (according to REGAUD, 1901) lipoidal droplets can be demonstrated. Benoit held that the granular type of secretory product dissolves in the apical cytoplasm and passes through the apical border of the cells in liquid form. He attacked the 'vesicular' theory of secretion elimination described by VAN DER STRICHT (1893), MYERS-WARD (1897), HAMMAR (1897), GURWITSCH (1902), JELENIEWSKI (1904), and REICHEL (1921). He believed, however, as did VIGNON (1901) that the vesicles seen on the surfaces of secreting cells represent spontaneous *post mortem* changes and claimed that they are never seen when fixation is good. MANEELY (1954) supported the older view that the vesicles seen at the surface of the epididymal cells, and those lying free in the lumen of the duct among the spermatozoa, are true evidence of secretion.

COLLERY (1949) also remarked on the dual nature of the epididymal secretion in the dog: the secretion globules consisting of an outer cytoplasmic sphere

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enclosing one or more granules. That the former is undifferentiated cytoplasm is shown by its staining reaction with eosin and light green; the granular core, however, may be of a lipoidal nature because of its reactions with silver and osmium and since it resists destaining after treatment with hot acid fuchsin. COLLERY (1949) also held the view that there is a relationship between the epididymal secretion and the 'middle piece bead' of the sperm cells (*vide* section 2).

LUDFORD (1925) described morphological changes in the epididymal epithelial cells during secretory activity and noted the occurrence of nuclear extrusions followed by nuclear budding. The result of these changes was the formation of secondary nuclei. Changes in the Golgi apparatus during secretory activity of the cells was described by LUDFORD (1925) and by NASSONOW (1923), who attributed the secretory activity of the cells to the influence of the Golgi apparatus on the cell cytoplasm. In this connection it is interesting to note that DALTON and FELIX (1953) have described the Golgi network in living epididymal cells of the mouse as seen by phase contrast microscopy.

BOMMER (1929), HAMPERL (1934) and SJÖSTRAND (1946) have reported the occurrence of autofluorescence in various tissues, including the epididymis. But it is not yet possible to assess the significance of the autofluorescent material seen in the epididymal epithelial cells nor to comment on its relationship with secretory mechanisms in the cells. Clearly, a detailed comparative study of the occurrence and distribution of autofluorescent material in the epididymis would be of great value.

5. Function and structure of the stereocilia

The ciliated nature of the apical cytoplasm of the epididymal cells been known since Becker's first description in 1856. KRAUSE (1876), HENRY (1900), FELIZET and BRANCA (1902) thought them vibratile; but AIGNER (1900), studying them *in vivo* in rats, pronounced them stationary, and in the same year, REICHEL named them *stereocilia*.

GURWITSCH (1902) was probably the first to suggest that *stereocilia* are involved in secretory phenomena and this view was endorsed by IKEDA (1906) and BENOIT (1926) who observed the progressive growth of the stereocilia during development, and their regression after castration. BENOIT (1926) regarded them simply as a differentiation of the apical cytoplasm which plays a rôle in the elimination of secretion products.

The difference between the stereocilia of the epididymis and the cilia of the *ductuli efferentes* were discussed by JORDAN and HELVESTINE (1923), who described a process of ciliogenesis in the *ductuli* of young rats. They regarded the epididymal stereocilia as 'rectilineal plates' and as 'spongioplastic extensions of the cytoplasm forced into rectilineal membranes by the cytodistal accumulation

of escaping material'. This may be true, but the morphology of the stereocilia seen by phase-contrast microscopy does not lend itself to the view that they are so fortuitously formed (personal observation).

Since the beginning of the 20th century the problem of ciliogenesis in cells has been linked with the problems of amitosis and the functions of the centrosome. Both HENNEGUY (1896) and LENHOSSEK (1898) denied the presence of a centrosome in ciliated epithelial cells, and from this argued that such cells could not divide mitotically. SAGUCHI (1917) however, disputed the Henneguy-Lenhossek theory and stated that a centrosome can be detected in ciliated cells in both invertebrates and vertebrates, that the sole method of division of ciliated cells is by mitosis in invertebrates and by amitosis in vertebrates, and that the ciliary apparatus is produced by the differentiation of mitochondria, the centrosome taking no part on its formation. However, the possible functions of the stereocilia seem more important at the present time than the finer points of argument as to their origin.

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SHAVER (1954) in an important article described the ability of stereocilia to remove india ink particles from the lumen of the rat epididymis. It had been known that Trypan blue injected beneath the *tunica albuginea* of the testis passes into the lumen of the seminiferous tubules and ultimately reaches the *ductus epididymis* whence it enters the epididymal cells (MASON and SHAVER, 1952). Whilst no comparison can be drawn between experiments with Trypan blue and those with india ink, SHAVER'S (1954) paper provides valuable evidence of dual polarity in the epithelial cells, the stereocilia having a 'sifting' action on the cellular debris in the upper portion of the epididymis.

6. The influence of castration on the epididymal tube

BENOIT (1926) gave the first detailed description of the effects of castration on the epididymis, and reported that in the mouse, guinea-pig and rabbit, bilateral castration produced rapid involution. The diameter of the epididymal canal and the height of the epithelial cells diminished rapidly after the operation, accompanied by a marked reduction, and ultimate cessation, of secretory phenomena. Unilateral castration on the other hand had little or no effect on the surviving epididymis on the unoperated side.

At the same time it was observed that many epithelial cells, especially in the *caput epididymis,* contained fuchsinophil 'sphérules hyalines'. BENOIT (1926) stated that these sphérules were certainly not secretion granules and suggested that they might represent degeneration of a portion of the cytoplasm; or might be material normally transformed by the epididymal epithelium which remains

unused on account of the cessation of the secretory activity. But whilst BENOIT'S (1926) observations regarding epididymal involution after bilateral castration are perfectly correct, other explanations of the 'sphérules hyalines' are possible. MANEELY (1958), using rats, repeated Benoit's experiments and reported that the secretory activity of the cells is not completely abolished. Six months after the operation diastase-resistant polysaccharide complexes (PAS-positive material) could still be demonstrated in the epithelial cells, especially in the lower *caput epididymis;* and similar PAS-positive material could be demonstrated in the lumen of the tube, mainly in the lower *cauda.*

During the involution of the epididymis pseudostratification of the epithelium occurred, and it was suggested that the appearance of conglomerations of material in the basal layers (Benoit's 'sphérules hyalines') might be due to the continuation of secretory activity of low intensity in the epithelial cells, some of which lose their connection with the lumen of the tube. Secretion which is unable to escape thus accumulates in the cytoplasm. An explanation of the continued secretory activity might be found in nervous control; but the part played by the nervous system in epididymal secretion is not known. MITCHELL (1935) and KUNTZ and MORRIS (1946) have described the innervation of the testis and epididymis; but the author has not been able to find any reports on nervous function in the epididymis. One possible explanation may be that the cells possess an intrinsic inherited secretory ability which normally becomes enhanced by the stimulating action of androgens.

Since the epithelial cells of the normal rat epididymis contain polysaccharide complexes which seem to be secreted into the lumen of the tube, it may be that they provide a source of 'structural' mucoproteins whose degree of polymerization may influence the downward movement of the spermatozoa. This might be achieved by altering the physical state of the seminal plasma (c.f. GERSH and CATCHPOLE, 1949).

MANEELY (1958) also reported that in some of the rats examined three weeks after bi-lateral castration, large caudal cysts had developed which were filled with spermatozoa. In other castrated rats of the same group no caudal cysts had formed and the caudal region was empty although some spermatozoa could be found in the *ductus deferens.* A possible explanation of this varied response three weeks after the operation rests in the observation reported by MARTINS and DO VALLE (1928) that whilst the *ductus deferens* in intact rats shows little or no spontaneous movements, rhythmic deferential contractions occur in a proportion of rats after castration. If castration affects contractility of the *ductus deferens* as a whole it may well involve the musculature of the intra-epididymal portion of the *ductus deferens* in a proportion of the operated rats. In such instances the *cauda epididymis* would be 'milked' and become empty of spermatozoa; but in those cases in which contractility of the *ductus deferens* is not augmented, caudal cysts filled with spermatozoa might develop. It is interesting to

note that Chevrel, Bodin and LeRoy (1941) reported the occurrence of similar caudal cysts filled with spermatozoa in rabbits treated with folliculin but at present there is no explanation as to the precise mechanism of their development, or whether they can be regarded as analogous to the cysts described above.

Bilateral castration has been reported to result in the disappearance of alkaline glycerophosphatase from the male genital tract of the rat (Soulairac and Thibault, 1948). On the other hand Bern and Levy (1952) reported, among other matters, that some phosphatase activity remains even five months after the operation, citing Nava and Fiore (1949) in support of their observations. This was confirmed by Maneely (1958) who noted that, although reduced in activity, alkaline phosphatase was demonstrable in the epididymis of rats castrated for six months. This reduction in acitivity was judged by comparing the incubation times at which the reaction for alkaline phosphatase could be elicited in sections prepared from normal and from castrated rats.

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7. Conclusions

1. Whilst it would seem to have been established that the spermatozoa in mammals undergo a 'ripening process' as they descend the epididymis, it is not yet possible to state whether or not the factors governing the 'ripening' process are intrinsic to the spermatozoa or reside in the epididymal epithelium surrounding them. It is clear that spermatozoa obtained from different anatomical regions of the epididymis possess differing biological properties; but the significance of those properties is not yet clearly understood.
2. Structural differences in the various anatomical regions of the epididymis have been established beyond doubt, including differences in the morphology and functions of the epithelium and of the stereocilia, and differences in the amount of smooth muscle surrounding the tube.
3. Regional histochemical differences also seem to have been established, comprising differences in the amount and distribution of diastase resistant polysaccharide complexes, and of alkaline glycerophosphatase.
4. Bi-lateral castration results in a rapid involution of the epididymis; but there is some evidence that the epithelium continues to secrete (at a low level of intensity) in the absence of testicular androgens. Polysaccharide complexes and alkaline glycerophosphatase can be demonstrated in the epididymis six months after bilateral castration.
5. The significance of alkaline glycerophosphatase and of polysaccharide complexes in epididymal physiology are not known; but it has been suggested here that a possible rôle for the polysaccharide complexes may be to provide a source of 'structural' mucoproteins whose degree of polymerization may

influence the downward movement of spermatozoa by altering the physical state of the epididymal seminal fluid.

6. Much more experimental work on the epididymis is required before answers to many of the unsolved questions concerning its rôle in male reproductive physiology can be attempted. The comparative histology, histochemistry and physiology of the epididymis over a wide range of animals, including a survey of epididymal function in hibernating species is an urgent necessity.

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Note: Some of the older references given below are now difficult to trace. Where I have not seen the original I state by whom it is quoted.

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Das Gehirn der Chinchillas und vergleichend-anatomische Betrachtungen mit verwandten Nagerarten (Rodentia, Hystricomorpha)*

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Inhalt

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Einleitung, Material

Von der vorwiegend nächtlichen Lebensweise der Chinchilla schreibt BREHM, „dass sie die scheinbar kahlen Felsenwände der Anden beleben, sich ungemein lebhaft und schnell bewegen, mit überraschender Leichtigkeit klettern sie an den Wänden hin und her, sie steigen sechs bis zehn Meter senkrecht empor mit einer Gewandtheit, dass man ihnen mit dem Auge kaum folgen kann. Je zerklüfteter die Wände, um so häufiger werden sie von den Chinchillas bewohnt" ...

Die Superfamilie Chinchilloidea (KRAGLIEVICH, 1940) hat in der einzigen Familie Chinchillidae 10 Gattungen. Davon sind 7 fossil und 3 sind rezent: Lagostomus (BROOKES, 1829), Lagidium (MEYEN, 1833) und Chinchilla (BENNETT, 1929). Die Gattung Chinchilla umfasst lediglich zwei Arten: Chinchilla *laniger* (MOLINA, 1782) und Chinchilla *brevicaudata* (WATERHOUSE, 1848). Ihre geographische Verbreitung ist auf die Anden beschränkt, von Peru bis Chile und Argentinien.

Trotz der interessanten Lebensweise und der vergleichend-anatomischen Bedeutung liegen über das Zentralnervensystem der Chinchillas keine Angaben vor.

* Aus dem Hirnanatomischen Institut (Prof. E. GRÜNTHAL) der psychiatrischen Universitätsklinik Waldau/Bern (Prof. M. MÜLLER).

Im folgenden soll eine makroskopische Beschreibung der Gehirne beider Arten erfolgen. Im zweiten Teil der Arbeit sind in den Grundzügen mehrere Arten von Hystricomorpha vergleichend-anatomisch untersucht. Die Chinchillas bekamen wir lebend aus der Zucht M. Zettl in München. Für einen Teil des vergleichend-anatomischen Materials sind wir den Herren Prof. D. Starck, H. Spatz und W. Krücke sehr dankbar.

BESCHREIBUNG DER GEHIRNE

Superfamilie: Chinchilloidea
Familie: Chinchillidae
Gattung: Chinchilla

Chinchilla laniger (Molina)

(Abb. 1, 3)

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Das von uns untersuchte Exemplar war 5 Jahre alt. Die Körpermasse sind:
T 1126 — ♂

Körpergewicht	425 gr	Ohrbreite	55 mm
Schnauze-Steisslänge	240 mm	Augenspalte	12 mm
Kopflänge	75 mm	Augendurchmesser	14 mm
Kopfbreite	55 mm	Schnauzborsten	110 mm
Schwanzlänge	135 mm	Hinterfusslänge	64 mm
Ohrlänge	70 mm		

Das Hirngewicht beträgt nach eintägiger Formolfixation *6,4 gr*. Das Verhältnis Hirngewicht (= 1) zum Körpergewicht ist *1 : 66*. Von oben betrachtet sind die Bulbi olfactori kaum vom Frontalpol überdeckt. Beide Grosshirnhemisphären zusammen haben eine rautenförmige Gestalt. Jede Hemisphäre ist dreieckig mit deutlicher Bildung von Occipitalpolen. Die dorsale Mantelfläche ist glatt, lediglich einige Millimeter rostral von den Occipitalpolen findet sich beidseits eine kurze längsverlaufende echte Furche, die ein feines Gefäss enthält. Die Lamina quadrigemina ist bei dorsaler Betrachtung nicht sichtbar, sie wird, wie der Medianschnitt zeigt (Abb. 3), vom Gross- und Kleinhirn überlagert. Das Kleinhirn hat, dorsal gesehen, eine annähernd rechteckige Form mit abgerundeten Ecken und ist breiter als lang. Es trägt gut entwickelte Paraflocculi, die kurz gestielt sind und deren Achsen leicht schräg kaudal gerichtet sind. Der Sulcus paramedianus ist im dorso-caudalen Bereich tiefer gezeichnet als im vorderen dorsalen Abschnitt. Die Verhältnisse im Wurm sind in der Abb. 3 schematisch dargestellt. Seitlich betrachtet fällt die starke Entwicklung des Kleinhirns in die Höhe auf. Es überschreitet die dorsale Ebene der Grosshirnhemisphären, die steil frontalwärts abfällt. Die Fissura rhinalis ist seitlich und ventral vom Frontalpol deutlich tief

Abb. 1. Gehirn von *Chinchilla laniger,* dorsal, basal und lateral gesehen.

gezeichnet, sie erscheint hingegen im Bereich der orbitalen Aushöhlung der Hemisphäre verwachsen, und wird im temporalen Gebiet wieder scharf konturiert. Sie kann von der Seite bis zu den Occipitalpolen verfolgt werden. Der Lobus pyriformis wölbt sich stark oralwärts vor; ein grosser Teil des Palaeocortex ist lateral sichtbar. Es fällt auch bei der Lateralansicht die starke Knickung der Medulla spinalis an der Uebergangsstelle zur Medulla oblongata auf. Basal betrachtet erscheint der Palaeocortex stark entwickelt. Die Tracti optici sind mehr quer gerichtet. Die Pars oralis tuberis ist kurz, die Hypophyse ist zwischen beiden Nervi tri-

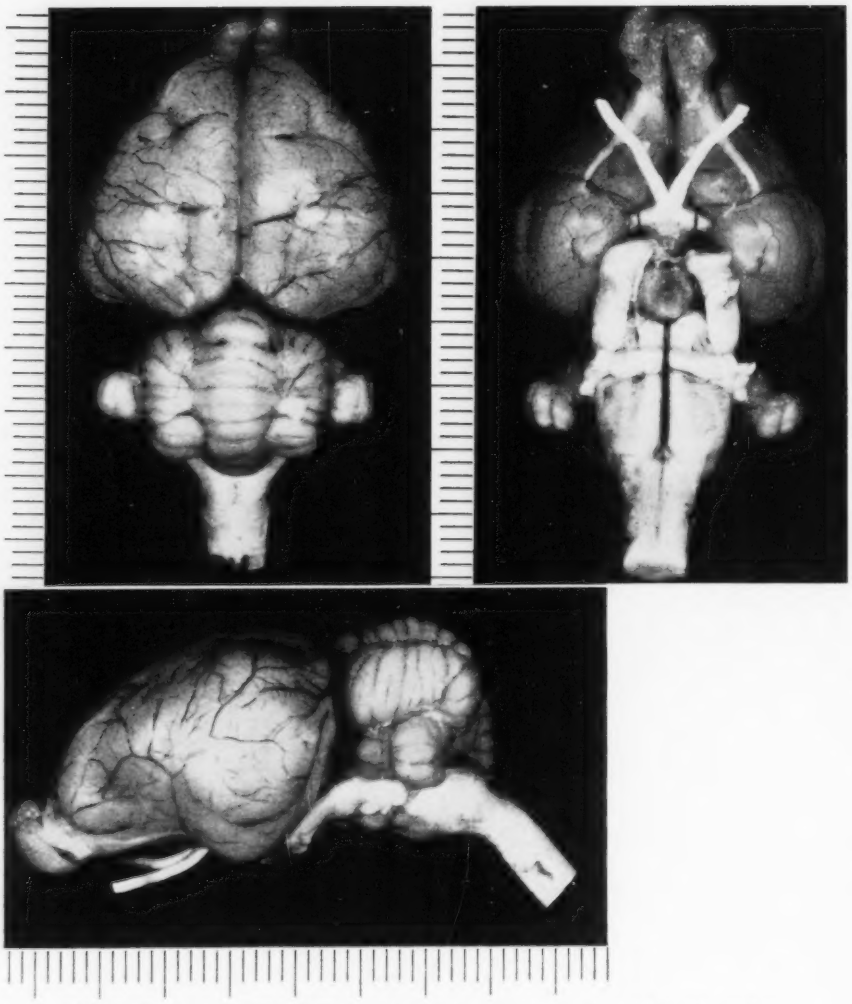

Abb. 2. Gehirn von *Chinchilla brevicaudata*, dorsal, basal und lateral gesehen.

gemini längs gezogen und überragt kaudal den freien Brückenrand etwas. Die Verhältnisse in der Brücke und der Med.oblongata sind aus den Abbildungen ersichtlich.

Chinchilla brevicaudata (WATERHOUSE)

(Abb. 2, 3)

Das von uns untersuchte Männchen war 4 Jahre alt. Die Körpermasse sind:
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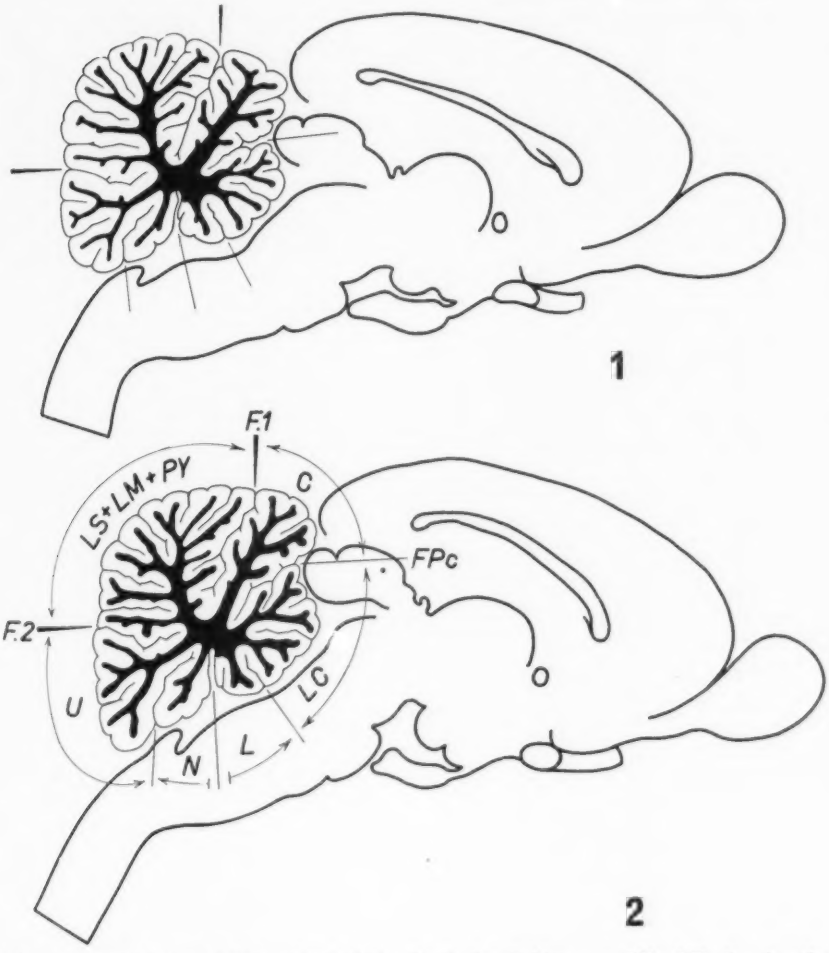

Abb. 3. Schematischer Mediansagittalschnitt durch das Gehirn von *Chinchilla brevicaudata* (*1*) und *laniger* (*2*). Im Kleinhirn: F_1 = Fissura prima, LS, LM, Py = Lobulus simplex, medius, Pyramis, F_2 = Fissura secunda, U = Uvula, N = Nodulus, L = Lingula, LC = Lobulus centralis, FPc = Fissura praecentralis.

Körpergewicht	520 gr	Ohrbreite	43 mm
Schnauze-Steisslänge	260 mm	Augenspalte	12 mm
Kopflänge	70 mm	Augendurchmesser	16 mm
Kopfbreite	40 mm	Schnauzborsten	110 mm
Schwanzlänge	105 mm	Hinterfusslänge	60 mm
Ohrlänge	40 mm		

Von oben gesehen bestehen im Grosshirn kaum nennenswerte Unterschiede gegenüber Chinchilla laniger. Hingegen sind die Paraflocculi des Kleinhirnes

etwas schlanker als bei Chinchilla laniger. Basal betrachtet erscheint die Hypophyse rundlicher als bei Ch. laniger; es fallen hier auch die schlankeren Paraflocculi auf. Bei der Seitenansicht sind in beiden Arten sehr geringe Unterschiede vorhanden, die jeglichen taxonomischen Wert entbehren. In der Tabelle 1 sind die Hirnmessungen angegeben.

VERGLEICHENDER TEIL

Auser den Chinchillas standen uns aus der Subordnung der Hystricomorpha Feuchtpräparate der Gehirne folgender Arten zur Verfügung:

1 Cavia porcellus	Familie Caviidae
2 Dolichotis patagona	
3 Hydrochoerus hydrochaeris	Familie Hydrochoeridae
4 Cuniculus paca	Familie Dasyproctidae
5 Dasyprocta aguti	
6 Dasyprocta azarae	
7 Lagostomus viscacia	Familie Chinchillidae

Die Tabelle 1 fasst die gesamten Messungen dieses Vergleichsmaterials und der Chinchillagehirne zusammen.

HIRNGEWICHT, KÖRPERGEWICHT

Soweit wir Angaben über das Körpergewicht sammeln konnten, sind diese in der Kurve Abb. 8 (die Arten sid 1 bis 9 systematisch geordnet) als Quotient Hirngewicht (= 1) zum Körpergewicht angegeben. Dadurch gewinnen wir über den Cerebralisationsgrad* einer Art eine *annähernde* Orientierung, da bekanntlich das Körpergewicht mit dem hirnunabhängigen Fettgewebe eine in einem bestimmten Bereich schwankende Grösse ist. Hoch cerebralisiert sind Cavia porcellus und beide Chinchilla-Arten; in einen mittleren Bereich der Cerebralisation fallen Cuniculus paca, die Gattung Dasyprocta und Lagostomus. Dann folgt Dolychotis patagona mit einem Quotienten von 1 : 220. Hydrochoerus hydrochaeris ist, als grösster Nager der Welt mit einem Quotienten 1 : 393 (nach WEBER) am niedrigsten cerebralisiert. In unserer Reihe weisen Chinchilla und Hydrochoerus die extremen Werte auf.

Grosshirn

Unter den hier untersuchten Arten finden wir solche mit einem praktisch lissencephalen Gehirn (Cavia, Chinchilla), andere mit einem stark gyrierten (Dolichotis, Hydrochoerus), ferner Uebergänge zwischen diesen Extremen. Dolichotis

* darunter verstehen wir die Beziehung Hirnmasse zur Körpermasse.

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Abb. 4. Gehirn von *Cavia porcellus,* dorsal, basal und lateral gesehen.

und Hydrochoerus sind unter den Nagetieren am stärksten gyriert. Ein Problem, das dabei auftaucht, ist die Beziehung zwischen Furchung und Körpergrösse, die Korrelation, die zuerst DARESTE bei vielen Tiergruppen festgestellt hatte. Nehmen wir als annäherndes Aequivalent der Körpergrösse das Gewicht des Tieres oder die Körperlänge, dann sehen wir bei den untersuchten Arten, dass eine direkte Korrelation zwischen Furchenbildung und Körpergrösse besteht. Sie geht auch dem Grade der Cerebralisation parallel: glatte Gehirne gehören hochcerebralisierten, kleineren Arten (Chinchilla, Cavia), und umgekehrt gefurchte Gehirne niedrig cerebralisierten, grösseren Arten (Dolichotis, Hydrochoerus).

Bei der Betrachtung der Form der Grosshirnhemisphären glaubten wir mit den von SCHNEIDER für das Chiropterengehirn entwickelten Winkelmessungen eine

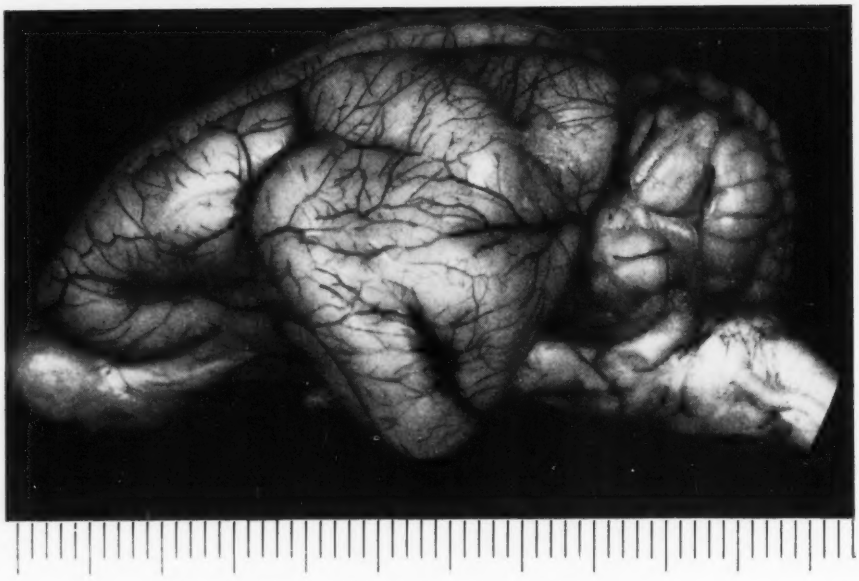

Abb. 5. Gehirn von *Dolichotis patagonica*, lateral gesehen.

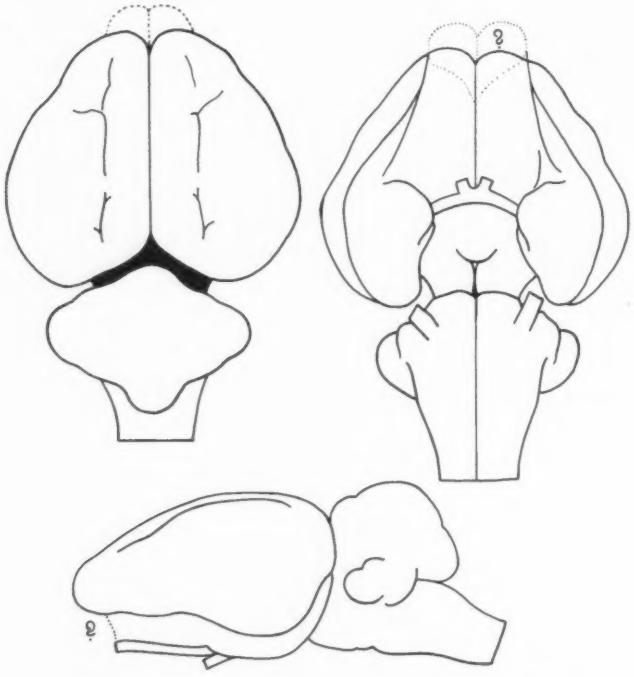

Abb. 6. Gehirn von *Cuniculus paca* (schematisch).

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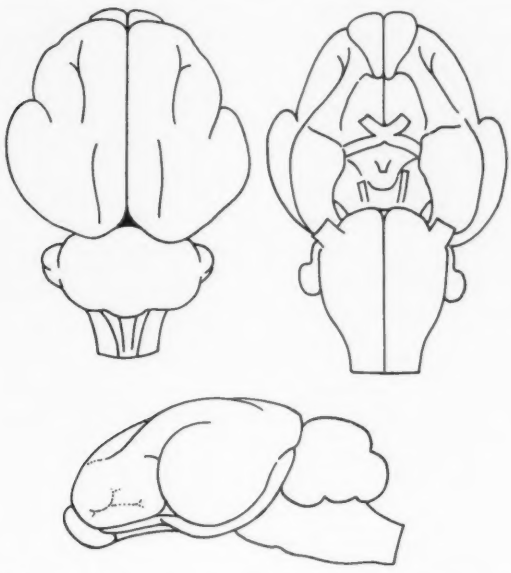

Abb. 7. Gehirn von *Lagostomus viscacia* (schematisch).

Korrelation zwischen der Systematik und bestimmten Stauchungen und Ausladungen der Grosshirnhemisphären finden zu können. Ohne diese Messungen hier speziell anzuführen, hat sich bei den hier untersuchten Formen gezeigt, dass diese Winkel Art- oder höchstens Gattungseigenschaften sind, die aber keine Beziehungen mit höheren systematischen Einheiten aufzeigen lassen. Wir beabsichtigen auf diese Methode zurückzukommen, wenn wir ein umfangreiches Material besitzen.

Verfolgen wir die hier zum Teil abgebildeten Gehirne unserer Reihe, fällt die leicht durchführbare Homologisierung der Furchen auf. Die *Fissura rhinalis* (palaeo-neocorticalis nach SPATZ) ist ein bei allen Arten konstantes Merkmal. Sie ist im rostralen Abschnitt, wo sie den Bulbus und Tractus olfactorius flankiert (= Fissura rhinalis anterior), scharf gezeichnet und tief, wird undeutlich und verschwommen in der Frontalebene des Lobus pyriformis und ist kaudal davon deutlich zu sehen (= Fissura rhinalis posterior). Lediglich beim Gehirn von Cuniculus paca erscheint sie ohne Unterbrechung von rostral nach kaudal durchgezogen.

Die *Fissura Sylvii* ist bei Cuniculus paca nicht vorhanden. Die Gehirne von Dasyprocta, Chinchilla und Cavia sind seitlich in Form einer „Vallecula" mehr oder minder stark eingedellt. Viscacia, Hydrochoerus und Dolichotis zeigen eine deutliche Furche, der die Bezeichnung Fissura Sylvii zweifelsohne gebührt.

Auf der dorsalen Hemisphärenfläche verläuft, der Mantelkante parallel, eine weitere Fissur. Sie ist bei Chinchilla nur im kaudalen Drittel der Hemisphäre

Hirnmessungen

(Tabelle 1)

Tierangaben	1	2	3	4	4[1]	5	6	7	8	9
Länge des Grosshirns	25	44	59	33	36	36	36	30	23	24
Breite des Grosshirns	25	46	64	42	44	35	33	32	25	27
Höhe des Grosshirns	14	29	33	23	24	24	24	17	15	18
Länge des Kleinhirns	13	19	22	22	24	19	15	16	14	15
Breite des Kleinhirns mit Paraflocculi	15	32	38	28	30	25	25	23	22	23
Breite des Kleinhirns ohne Paraflocculi	13	23	38	23	—	—	21	18	16	16
Höhe des Kleinhirns mit Brücke	15	26	31	23	24	21	21	19	17	17
Länge der Brücke	3	6	8,5	8	6,5	6	—	7,5	4	3,5
Breite den Brücke	9	16	34	19	18	14	14	18	10	12
Länge des Bulbus olfactorius	7	13	12	11	10,5	11	11	—	8	8
Breite des Bulbus olfactorius	5	8	4	7	7	5,5	5	—	4	4
Länge des Tractus olfactorius	8	12	23	9	12	10	11	8,5	8,4	9
Breite des Tuberculum olfactorium	4,5	7	10	8,5	10	7,5	7	5	5	6,5
Entfernung zwischen den Fissurae rhinales	21	27	40	33	37	27	28	24	21	23
Fissura rhinalis — Uncus pyriformis	7,5	11	13	11,5	12	10	11	8	8	8
Kleinste Entfernung zwischen den Unci pyriformes	6	8	17	10	12	7	6	9	6	6,5
Länge des Hypothalamus	7	10	14	10	10	10	11,5	8,3	6	7
Durchmesser des N. opticus	1	2,5	4	1,5	1,6	2,3	2	1,2	1	1
Durchmesser des N. trigeminus	4	5	—	5	6	3	—	5	3	3
INDEX $\frac{\text{Hypothalamuslänge}}{\text{Grosshirnlänge}}$	0,28	0,22	0,24	0,30	0,26	0,26	0,32	0,28	0,26	0,29
Hypophysenlänge	5	8	—	—	—	—	6	—	6	5
Hypophysenbreite	5	9	—	—	—	—	4,5	—	3,5	5

1 = *Cavia porcellus*	T 1117	Sammlung *Hirnanatomisches Institut Bern*
2 = *Dolichotis patagona*	T 1122	Sammlung *Hirnanat. Institut Bern*
3 = *Hydrochoerus hydrochaeris*	T IX/24/4	Sammlung *Max Planck, Institut Giessen*
4 = *Cunciculus paca*	IX/24/3	Sammlung *Max Planck, Institut Giessen*
4[1] = *Cunciculus paca*	(H)	Sammlung *Edinger Institut Frankfurt*
5 = *Dasyprocta aguti*	(I)	Sammlung *Edinger Institut Frankfurt*
6 = *Dasyprocta azarae*	—	Sammlung *Anatom. Institut Frankfurt*
7 = *Lagostomus viscacia*	IX/25/1	Sammlung *Max Planck, Institut Giessen*
8 = *Chinchilla laniger*	T 1126	Sammlung *Hirnanat. Institut Bern*
9 = *Chinchilla brevicaudata*	T 1125	Sammlung *Hirnanat. Institut Bern*

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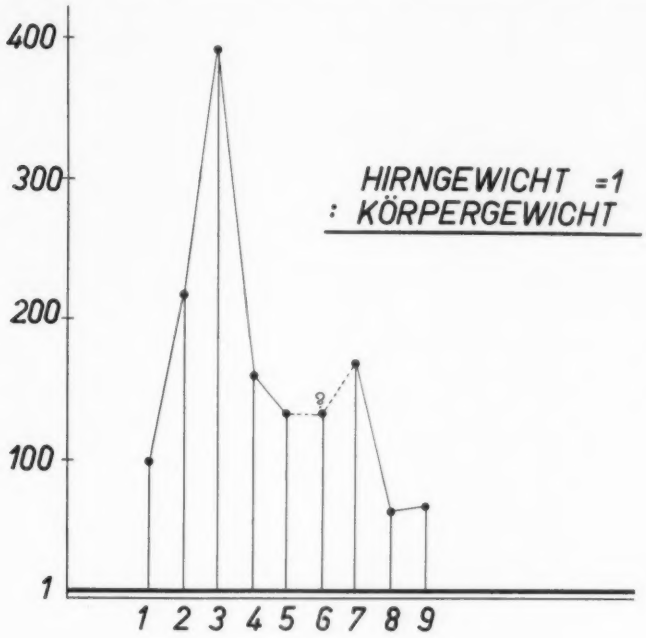

Abb. 8.

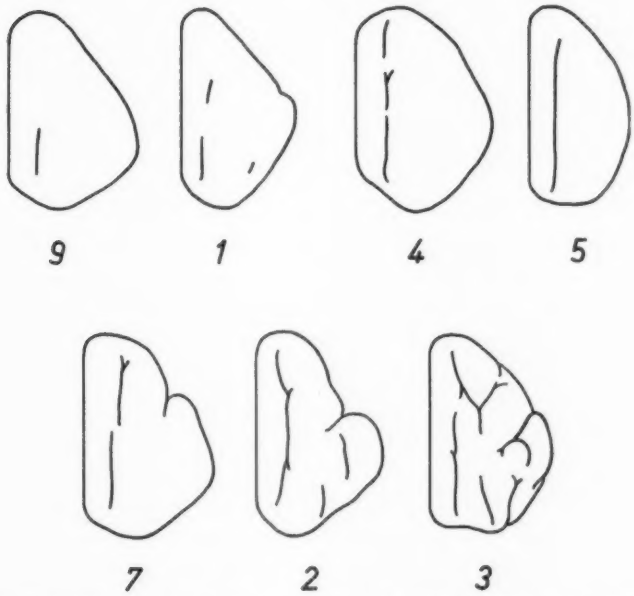

Abb. 9. Schematische Darstellung der Furchen auf der Dorsalfläche der Grosshirnhemisphäre. Die Hemisphären sind in der gleichen Grösse gezeichnet.

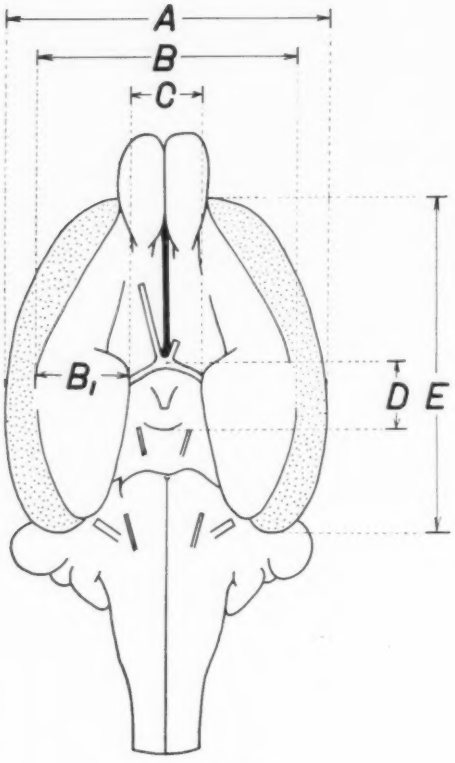

Abb. 10.

vorhanden. Bei Cavia fügt sich im mittleren Bereich ein kurzes Segment hinzu, das einige Millimeter lateraler vom ersten verläuft. Bei Viscacia reichen die beiden Segmente fast zum frontalen und occipitalen Pole, wobei hier auch das rostrale Segment mehr lateral liegt. Bei Cuniculus ist diese Parasagittalfurche mit leichten Unterbrechungen fast kontinuierlich. Deutlicher wird sie beim Aguti-Gehirn und am tiefsten imprimiert ist sie auf der Grosshirnrinde von Dolichotis und Hydrochoerus. Diese beiden Arten weisen ausser den Fissurae rhinalis, Sylvii und parasagittalis noch weitere kleine Furchen auf, die in einem weiteren Beitrag eingehend beschrieben werden sollen. Die Abb. 9 zeigt die progressive Zunahme der Furchung in den 9 Arten, deren Gehirne hier gleich gross gezeichnet sind.

Die Fissura rhinalis trennt als palaeo-neocorticale Grenze den Palaeo- vom Neocortex ab. Der Paleocortex kommt immer basal oder laterobasal zu liegen und kann bei den hier untersuchten Hystricomorpha niemals von dorsal gesehen werden. Wir haben, um eine Objektivierung der quantitativen Beziehungen zwischen

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Palaeo- und Neocortex zu gewinnen folgenden Index eingeführt, dessen Ableitung aus der Abbildung 10 ersichtlich ist:

$$\frac{B_1}{B_1 + \frac{A - B}{2}}$$

Es ist klar, dass dieser Index lediglich die maximale Ausdehnung des Palaeocortex und deren Beziehung zur Gesamthirnbreite im Bereich einer mittleren Frontalebene berücksichtigt. Palaeo-neocorticale *Volum*verhältnisse können annähernd mit der Gewichtsanalyse, exakt aber nur histologisch erfasst werden. Betrachtet man die Gehirne von Cavia oder Chinchilla und vergleicht sie von der Seite mit dem von Dolichotis, fällt sofort die progressive Senkung *neo*corticaler Gebiete im temporalen Bereich der Hemisphäre auf. Der Palaeocortex wird durch die Bildung eines neocorticalen Temporalpoles von der Seite allmählich verdeckt. Wenn man die Indices, nach der Systematik geordnet, graphisch darstellt (Abb. 11), sieht man, dass die einzelnen Werte an Arten und Gattungen gebunden sind und innerhalb einer Familie ziemlich stark variieren können (Cavia, Dolichotis!). Hydrochoerus und Dolichotis fallen an die untere Grenze der Kurve. Ordnet man die Werte in arithmetischer Folge, ergibt sich folgende Reihe:

(Tabelle 2)

Art	Palaeo-Neocortex-Index	Hypothalamus Index	Furchung
Chinchilla laniger	0,80	0,26	fast lissencephal
Chinchilla brevicaudata	0,80	0,29	
Cavia porcellus	0,78	0.30	
Cuniculus paca	0,74	0,30—0,26	schwach gyriert
Dasyprocta aguti	0,71	0,26	
Lagostomus viscacia	0,68	0,28	stärker gyriert
Dolichotis patagona	0,54	0,22	am stärksten gyriert
Hydrochoerus hydrochoeris	0,52	0,24	

Chinchilla hätte danach die stärkste palaeo-, Hydrochoerus die stärkste neocorticale Entwicklung. Jener ist, wie früher festgestellt, wurde hoch-, dieser schwach cerebralisiert. Eine direkte Beziehung ergibt sich zwischen dem Indexwert und dem Grad der Furchung. Je stärker gefurcht das Gehirn aussieht, desto geringer ist die relative Entwicklung des Palaeocortex und umgekehrt. Es wird

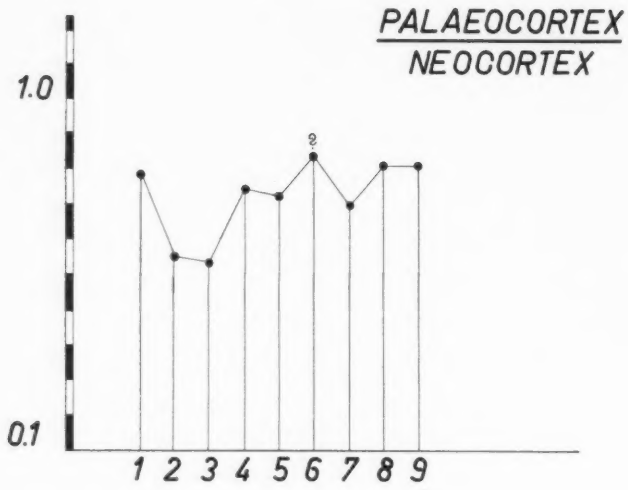

Abb. 11.

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interessant sein, diese aus der rein makroskopischen Betrachtung gewonnenen Tatsachen mit mikroskopischen Messungen später nachzuprüfen.

Hypothalamus

Zur Beurteilung dieses Hirngebietes kennen wir den Index Hypothalamuslänge: Grosshirnlänge (GRÜNTHAL), und man nimmt an, dass mit Zunahme des Neocortex eine Reduktion des Hypothalamus stattfindet. Tatsächlich weisen Hydrochoerus (siehe Tabelle 2) und Dolichotis extrem niedere Werte, Chinchilla und Cavia höhere Werte auf. Für die übrigen Arten geht der Indexwert der neocorticalen Entwicklung keineswegs parallel, wenn man den Palaeo-neocortex-Index als Mass der Entfaltung des Neocortex nimmt. Bei den individuellen Schwankungen der Hypothalamuslänge müssen immer Artserien verglichen werden und nur Mittelwerte sind dann brauchbar. Ein solches Material steht uns noch nicht zur Verfügung.

Kleinhirn

Ein Blick auf die Abbildungen 1, 4, 6, 7 zeigt uns das Persistieren einer gleichen Grundform des Kleinhirnes bei allen untersuchten Arten. Von oben betrachtet ist das Kleinhirn meistens rechteckig, mit abgerundeten Seitenpartien, wobei der Querdurchmesser meistens überwiegt. Eine Homologisierung der Einzelteile berei-

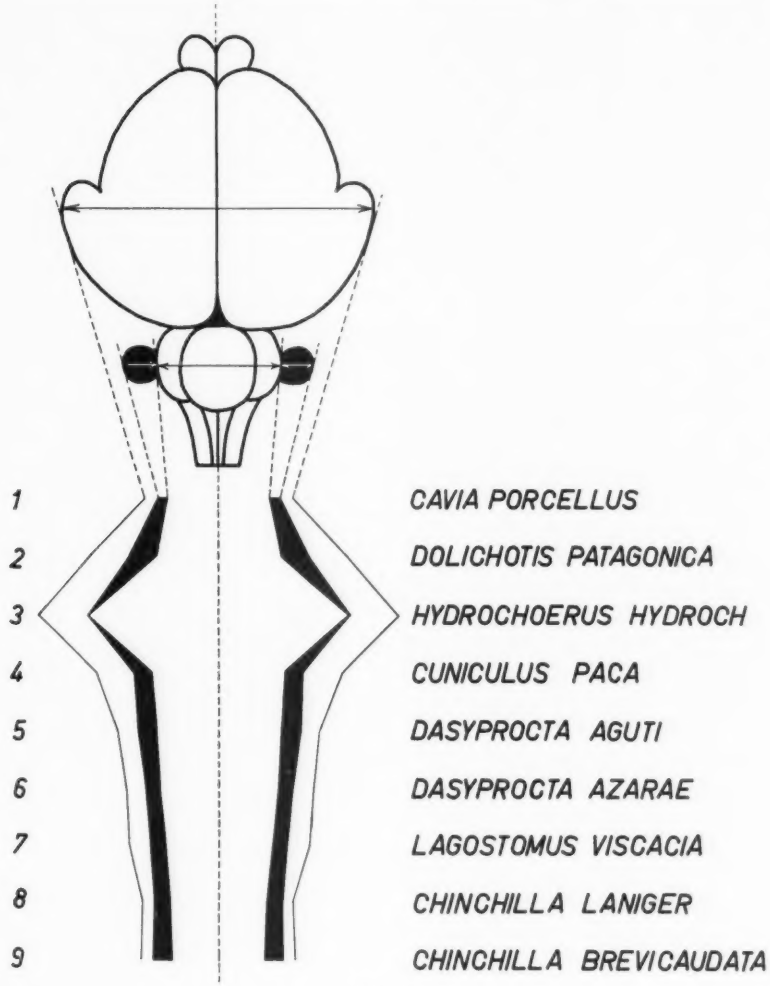

Abb. 12.

tet keine Schwierigkeiten. Der Sulcus paramedianus ist seicht, mehr dorso-caudal entwickelt. Auffallend sind die Schwankungen der Breite der Paraflocculi, wie es am besten das Schema Abb. 12 anschaulich macht. Das Schema zeigt die absoluten Werte der Breite des Grosshirnes und der des Kleinhirnes mit und ohne Paraflocculus. Bei keiner der untersuchten Arten ist das Kleinhirn breiter oder gleichbreit (siehe *Sciuromorpha*) wie das Grosshirn. Auch für die Kleinhirn-Grosshirnverhältnisse haben wir die Messwerte in Indexform ausgearbeitet. Wenn man vergleicht, erweist sich die Ausdehnung des Kleinhirns bei den verschiedenen

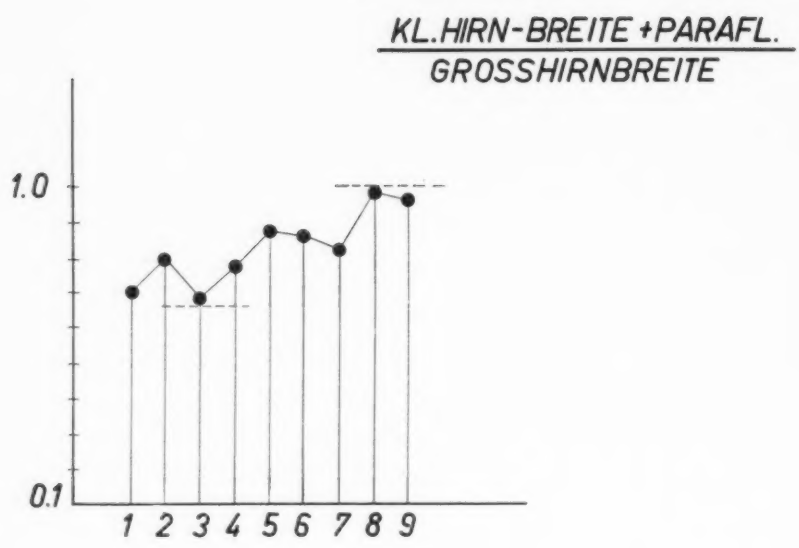

Abb. 13.

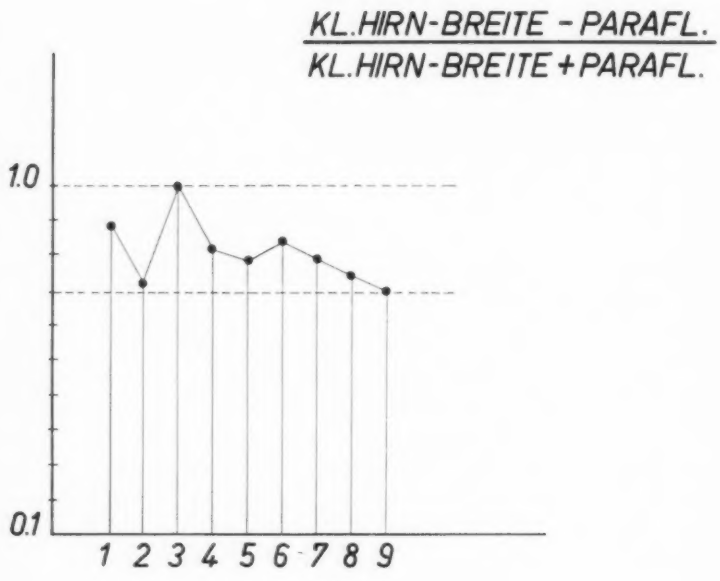

Abb. 14.

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Arten zum Grosshirn nicht proportional (Abb. 12, 13). Betrachtet man den Paraflocculus, so hat bei den Caviidae das Meerschweinchen einen kurzen, Dolichotis hingegen einen langen Paraflocculus. Bei Hydrochoerus ist dieser, bei einer allgemeinen Abnahme der Kleinhirndimensionen, noch weniger vorspringend. Die Dasyproctidae haben gut entwickelte Paraflocculi. Mit Chinchilla wird ein extremer Wert erreicht. (Kurve Abb. 14). Die Indices sind Ausdruck morphologischer Eigenschaften, die gattungs- oder artgebunden sind. Wir sehen in diesen Schwankungen den Ausdruck besonderer Artgestalten, die spezielle Funktionsmechanismen und Strukturen im zentralnervösen Organ erfordern. Denkt man an die Vielfalt der Formen der Motorik bei den hier in Frage kommenden Nagern, die Unterschiede zwischen einer Chinchilla mit ihrer vorzüglichen Kletterkunst, eines laufenden Meerschweinchens, der hüpfenden Mara und dem gemächlichen Capybara, dann wird es einleuchtend, dass bei der Phylogenese hier *art*gebundene Umweltsadaptationen und Spezialisationen in funktionell morphologischer Richtung vor sich gegangen sind.

Auch die innere Struktur des Kleinhirnes zeigt die progressive Differenzierungen (Abb. 15,3) von Arten mit kleinen Paraflocculi (Cavia) und Arten mit langen Paraflocculi (Dolichotis, Chinchilla).

Zusammenfassende Ergebnisse

Im ersten Teil der Arbeit erfolgt eine makroskopische Beschreibung des Gehirnes von *Chinchilla laniger* und *brevicaudata,* deren zentralnervöse Strukturen bisher nicht untersucht wurden. Diese werden im zweiten Teil vergleichend-anatomisch mit denen anderer Hystricomorpha (*Cavia, Dolichotis, Hydrochoerus, Cuniculus, Dasyprocta, Lagostomus*) verarbeitet, wobei hier vor allem quantitative Verhältnisse herangezogen werden. Es erweist sich in unserer Reihe dass Tiere mit grossem Körpergewicht im allgemeinen niedrig cerebralisiert sind und umgekehrt. Eine weitere Korrelation besteht zwischen Körpergrösse einerseits und dem Grade der Furchung anderseits, indem die grössten Tiere auch am stärksten gyriert erscheinen. Ueber die palaeo-neocorticalen Beziehungen kann man sich am makroskopischen Präparat mittels eines neuen Index orientieren. Eine direkte Beziehung besteht zwischen diesem Indexwert und dem Furchungsgrad: je stärker die Furchung im Grosshirn, desto geringer ist die relative Ausdehnung des Palaeocortex und umgekehrt. Die Ausdehnung des Kleinhirnes ist der des Grosshirnes bei den verschiedenen Arten nicht proportional. Unter Bewahrung der gleichen Grundform des Cerebellums ist die Länge des Paraflocculus grossen Schwankungen unterworfen. Es handelt sich hier um art- bzw. gattungsgebundene Merkmale welche Ausdruck einer besonderer Spezialisation sind.

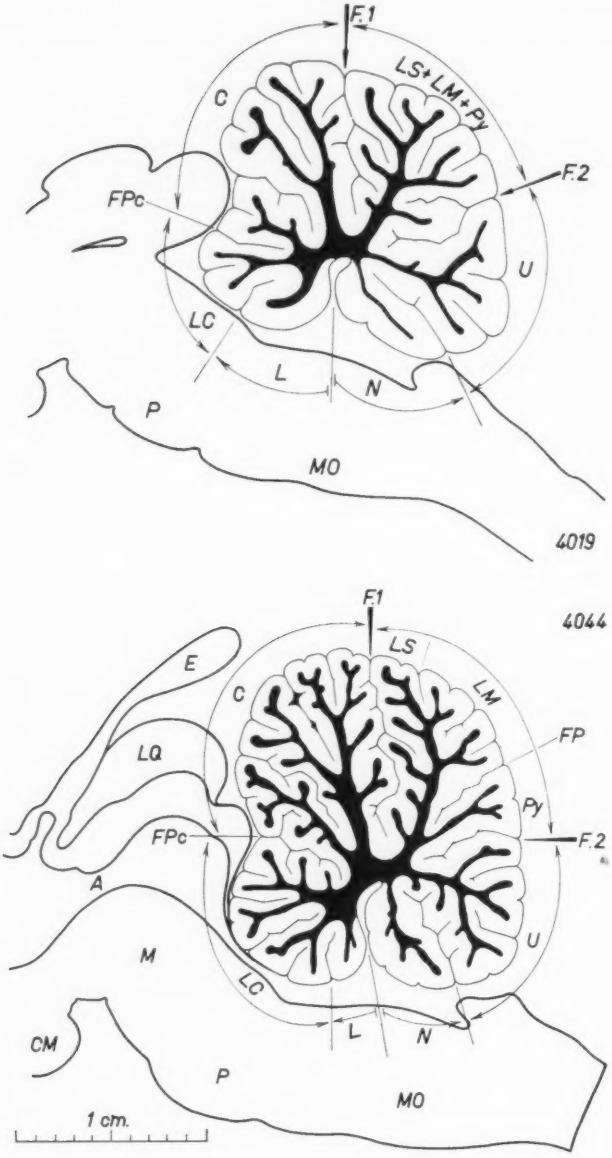

Abb. 15. Schematischer Mediansagittalschnitt durch das Kleinhirn von *Cavia porcellus* (oben) und *Dolichotis patagona* (unten). Für die Bezeichnungen siehe Abb. 3.

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Das Gehirn von *Dolichotis patagona* und *Hydrochoerus hydrochaeris*, nebst Betrachtungen über die endocraniellen Verhältnisse (Rodentia, Hystricomorpha)*

von

G. PILLERI

Inhalt

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Einleitung

Die erste brauchbare Beschreibung des Gehirnes von *Dolichotis patagona* verfasste BEDDARD (1892), wobei er sich aber nur auf die Darstellung der Furchen des Grosshirnes und deren Homologisierung mit denen anderer Hystricomorpha beschränkte. Das *Capybara*-Gehirn wurde zum ersten Male von C. DARESTE (1855) untersucht. Schon früher war M. DUVERNOY die für ein Nagetier ungewöhnliche Furchung der Hemisphären aufgefallen, und er vermutete eine Beziehung zwischen dieser und der Körpergrösse des Tieres. Auch DAUBENTON (siehe BUFFON: Histoire Naturelle) waren die Furchen des Capybara nicht entgangen. Die letzte Beschreibung von MADELEINE FRIANT (1954) behandelt vorwiegend das Furchenbild im Hinblick auf die Ontogenese.

In der folgenden Arbeit sollen diese alten Angaben revidiert und ergänzt werden. Es soll ferner auf die Eigentümlichkeiten des Schädelbinnenraumes eingegangen werden, welche offenbar wichtige morphologische Symptome zum Verständnis der Hirnevolution bei stärker gyrierten Nagetieren darstellen.

Ein junges Exemplar von *Dolichotis patagona* wurde uns von Prof. D. STARCK (Anatomisches Institut Frankfurt/a.M.) überlassen. Ein erwachsenes Exemplar derselben Art verdanken wir der Direktion des Hagenbeck-Zoo in Hamburg. Das hier beschriebene Gehirn von *Capybara* gehört der Sammlung des Max Planck-

* Aus dem Hirnanatomischen Institut Waldau/Bern (Schweiz)
(Leiter: Prof. E. Grünthal)

Institut für Hirnforschung Giessen (Prof. H. SPATZ). Die zum Studium des Endocranium verwendeten Schädel stammen aus dem Naturhistorischen Museum in Bern (Dr. W. KÜENZI). Den Institutleitern sprechen wir unseren ergebenen Dank aus. Die Endocranialausgüsse wurden mit einem kaltvulkanisierenden Silikonkautschuck (Giessmasse 56 + Härter T; WACKER) hergestellt.

Beschreibung der Gehirne

Familie: Caviidae
Subfamilie: Dolichotinae
Gattung: Dolichotis (DESMAREST, 1820)

Dolichotis patagona (ZIMMERMANN)

(Abb. 1—6)

T 1122 ♂ — Körpermessungen:

Körperlänge	65 cm
Hinterfusslänge	16 cm
Ohrlänge	10 cm
Ohrbreite	5 cm
Augenspalte	2 cm
Körperlänge + gestreckte Extremitäten	100 cm
Körpergewicht	5,65 kg

Das Hirngewicht beträgt *25,66 gr.* (nach WARNCKE: *30,5 gr.*). Das *Verhältnis Hirngewicht (= 1) zum Körpergewicht ist 1: 220. Dorsal* betrachtet (Abb. 1) sind die Grosshirnhemisphären dreieckig, frontal und occipital etwas abgerundet, lateral sehr stark ausladend. Am Ende der Medianfurche ist das distale Ende der Epiphyse (E) sichtbar. Die Bulbi olfactorii sind vom Frontalpol zum grossen Teil überdeckt. Paramedian, Occipital- mit Frontalpol verbindend, verläuft eine zickzack förmige ziemlich tiefe Furche (a). Sie endet einige Millimeter vor den Polen. Lateral von dieser Furche findet sich im occipito-dorsalen Bereich ein Grübchen (c), das zwischen zwei vorspringenden Rindengebieten liegt. Seitlich, ungefähr in der Mitte der Grosshirnhemisphären, verläuft schräg von medial nach lateral eine tiefe Furche die caudal um 90° abbiegt. Zwischen der temporalen und occipitalen Vorwölbung wird diese ganz seicht und verliert sich, die caudale Hemisphärenkante überschreitend, gegen das Mittelhirn. *Lateral* betrachtet ist die Bedeckung der Bulbi olfactori durch die neocorticalen Gebiete sehr augenfällig. Die Fissura rhinalis anterior ist bis zur Tractus olfactorius-Mitte deutlich gezeichnet, dann wird sie seicht, kaum angedeutet und wird in der Höhe

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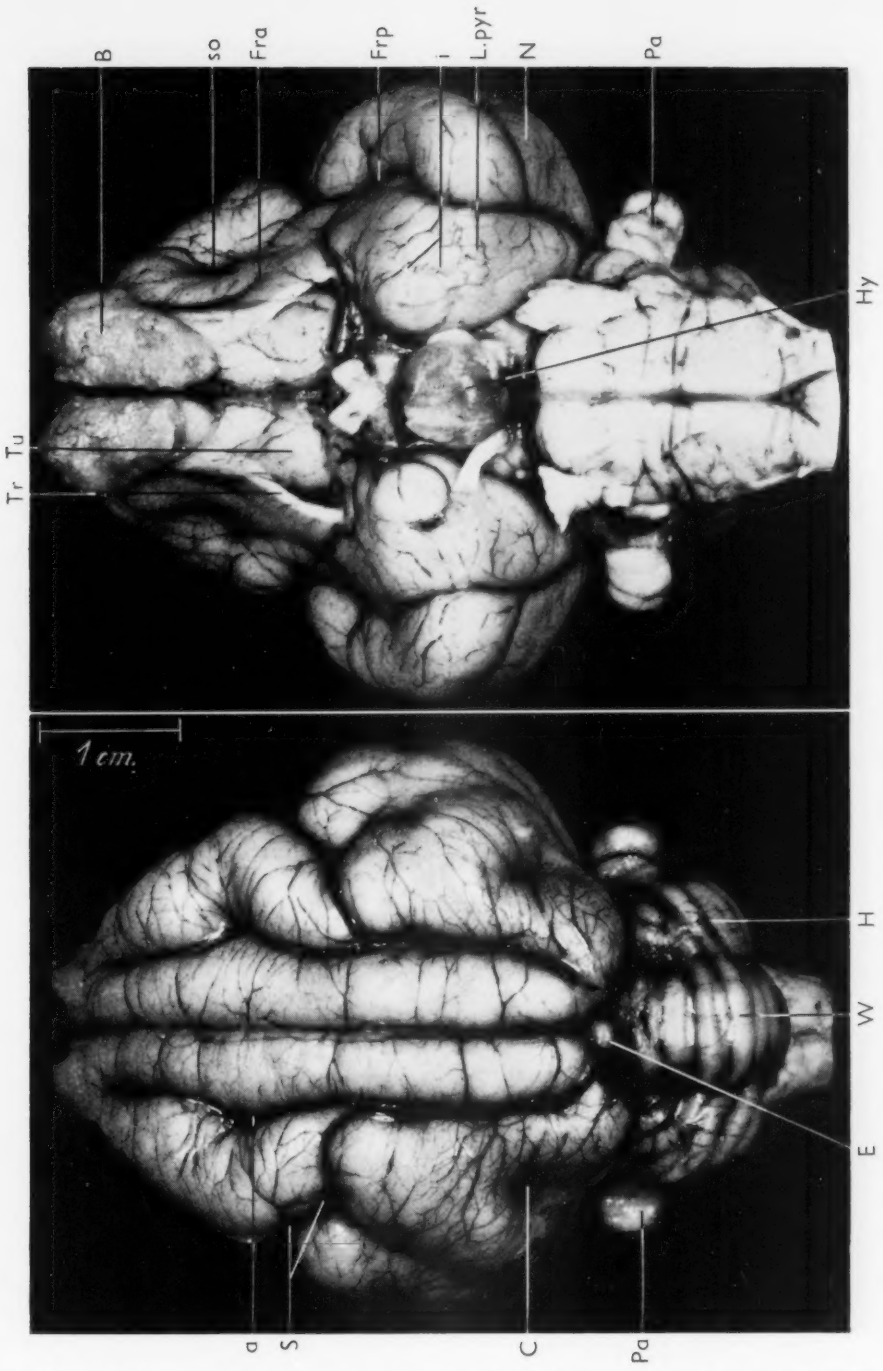

Abb. 1. Gehirn von *Dolichotis patagonica,* dorsal und basal gesehen: Pa = Paraflocculus, E = Epiphyse, W = Kleinhirnwurm, H = Kleinhirnhemisphäre, B = Bulbus olfactorius, Tr = Tractus olfactorius, Tu = Tuberculum olfactorium, Fra = Fissura rhinalis anterior, Frp = Fissura rhinalis posterior, L.pyr. = Lobus pyriformis, N = Neocortex, Hy = Hypophyse. Für die restlichen Buchstaben *siehe Text.*

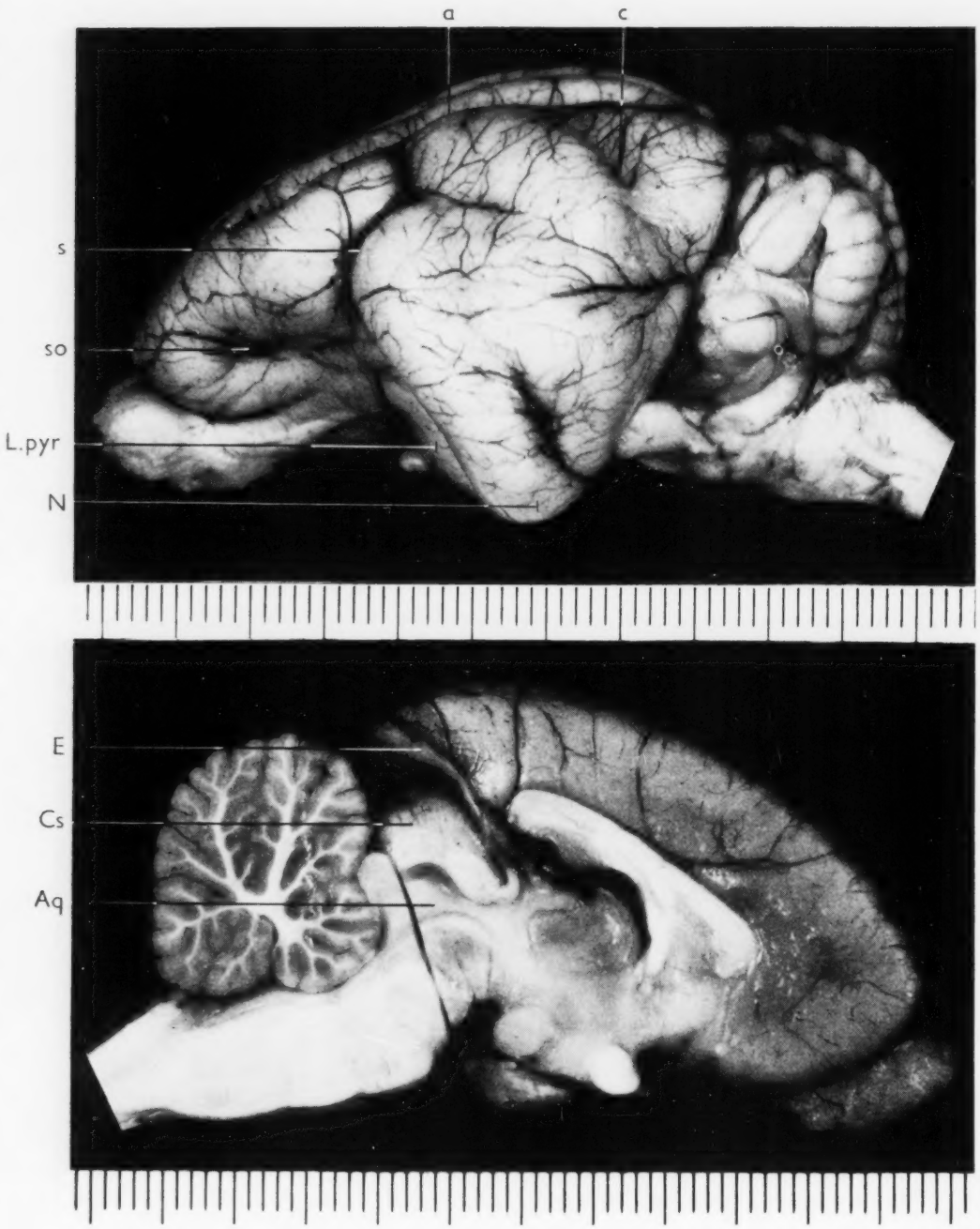

Abb. 2. Gehirn von *Dolichotis patagona,* lateral und medial gesehen: Cs = Colliculus superior laminae quadrigeminae, Aq = Aquaeductus Sylvii, E = Epiphyse. Übrige Buchstaben wie Abb. 1.

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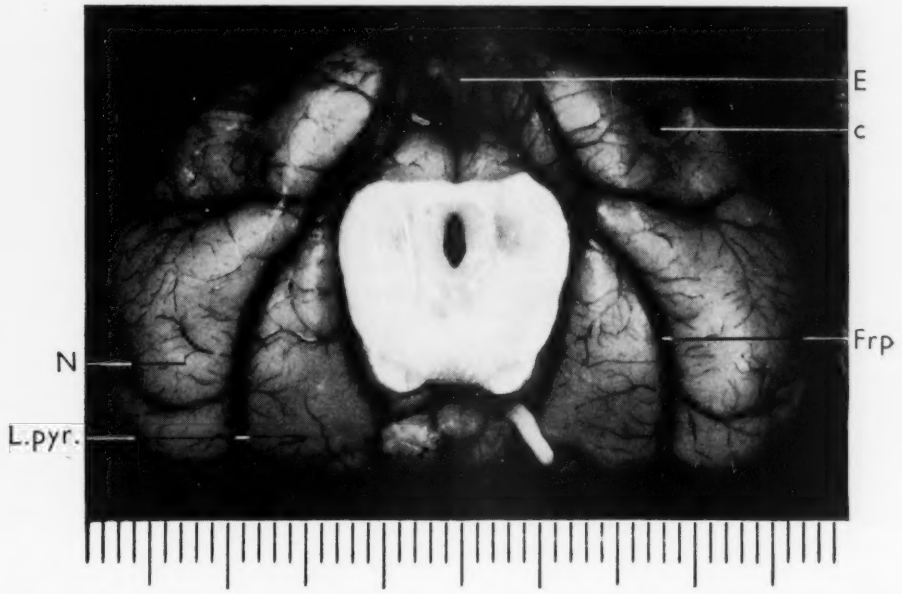

Abb. 3. Grosshirn von *Dolichotis patagona*, caudale Fläche: E = Epiphyse, Frp = Fissura rhinalis posterior, N = Neocortex, L.pyr. = Lobus pyriformis (Palaeocortex), c = siehe Text.

der Temporalpole wieder tief. Im orbitalen Gebiet, seitlich des Tractus olfactorius, findet sich ein längsverlaufendes Grübchen (so). Vom Lobus pyriformis ist nur der orale Abschnitt sichtbar. Dieser Teil ist hier steil zur Längsachse des Cerebrums gerichtet. Ein mächtig entwickelter Temporalpol wird von einer steil verlaufenden Furche durchzogen. Aus der Fissura rhinalis posterior bildet sich die Fissura Sylvii (s), die im S-förmigen Verlauf die dorsale Hirnfläche erreicht. *Basal* betrachtet sind die orbitalen, lateral vom Rhinencephalon liegenden Gebiete, schmal; die temporale Ausladung ist mächtig. Die Fissura rhinalis posterior ist tief gezeichnet, grenzt einen breiten Palaeocortex ab, der von einem fast gleich breiten neocorticalen Gebiet flankiert wird. Die palaeo-neocorticale Grenze (Fissura rhinalis posterior) beschreibt einen leichten, nach aussen konvexen Bogen und wendet sich im caudalen Cortexbereich gegen die Vierhügelplatte. Lateral vom Uncus pyriformis findet sich eine sehr schwach ausgebildete intrapyriforme kurze Furche (i), die ein Gefäss enthält. Die Tracti olfactorii sind schmal und leicht S-förmig gebogen. Die Tubercula olfactoria sind durch die orbitale Aussparung der Hirnbasis schief gelegen und springen sehr wenig vor. Die Tracti und Nervi optici sind gut enwickelt. Der Durchmesser der Augen beträgt *26 mm*. Vom Hypothalamus ist die Pars oralis tuberis kurz. Das Infundibulum geht in eine rundliche, flache Hypophyse über, die den freien Brückenrand nicht berührt und die Fossa interpeduncularis nicht ausfüllt. Teile der Pedunculi cerebri und die

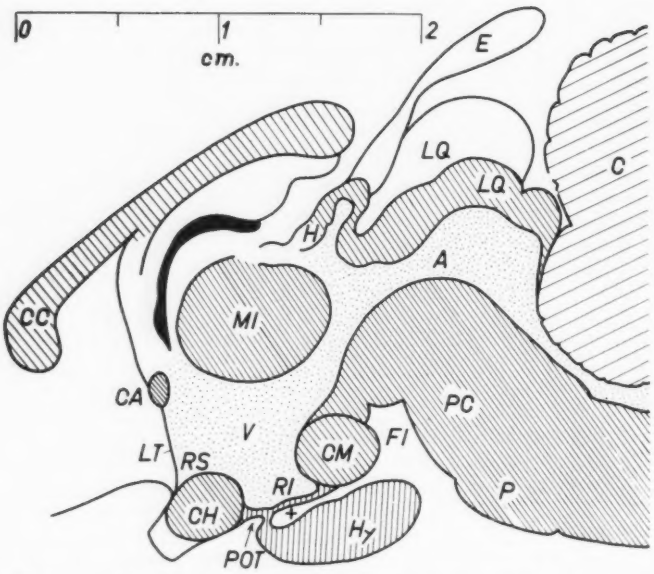

Abb. 4. Mediansagittalschnitt durch das Zwischenhirn von *Dolichotis patagona* (schematisch): E = Epiphyse, LQ = Lamina quadrigemina, C = Cerebellum, CC = Corpus callosum, H = Habenula, A = Aquaeductus Sylvii, MI = Massa intermedia, CA = Commissura anterior, V = III. Ventrikel, LT = Lamina terminalis, RS = Recessus supraopticus, RI = Recessus infundibuli, CH = Chiasma, POT = Pars oralis tuberis, PCT = Pars caudalis tuberis, Hy = Hypophyse, CM = Corpus mammillare, PC = Pedunculus cerebri, P = Pons, FI = Fossa interpeduncularis.

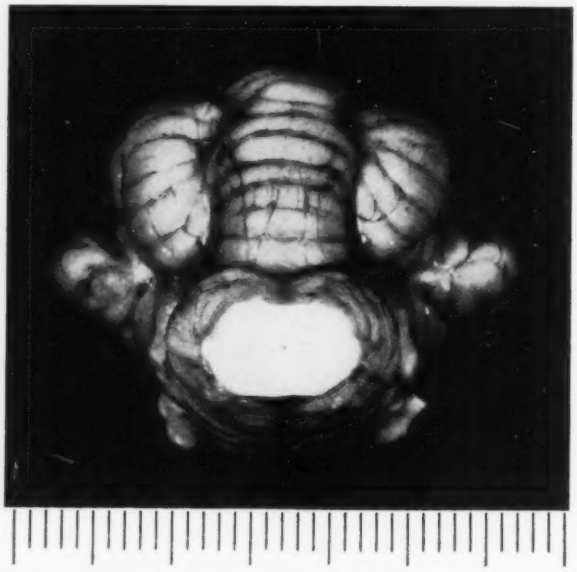

Abb. 5. Kleinhirn und Medulla oblongata von *Dolichotis patagona*, caudal gesehen.

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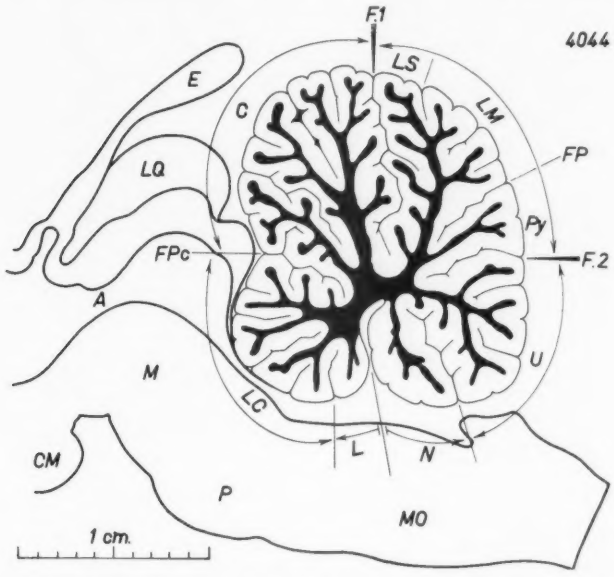

Abb. 6. Mediansagittalschnitt durch das Kleinhirn von *Dolichotis patagona:* E = Epiphyse, LQ = Lamina quadrigemina, A = Aquaeductus Sylvii, M = Mittelhirn, CM = Corpus mammillare, P = Pons, MO = Medulla oblongata. — L = Lingula, LC = Lobulus centralis, FPc = Fissura praecentralis, C = Culmen, F.1 = Fissura prima, LS = Lobulus simplex, LM = Labulus medius, FP = Fissura praepyramidalis, Py = Pyramis, F.2 = Fissura secunda, U = Uvula, N = Nodulus.

Abgangsstelle des Nervus oculomotorius sind basal sichtbar. Das Kleinhirn ist schmäler als das Grosshirn. Der Sulcus paramedianus ist schwach ausgebildet und nur caudo-dorsal stärker imprimiert (Abb. 1). Die Paraflocculi sind gut entwickelt, leicht gestielt und, caudal betrachtet, schräg nach oben gerichtet.

Mediansagittalschnitt: Die Epiphyse ist keulenförmig, 13,5 mm lang. Wieweit der Recessus in die Drüse hineinreicht wird die histologische Untersuchung aufzeigen. Wir haben ausser bei *Coelogenys paca* und *Hydrochoerus hydrochaeris* bei keinem der übrigen untersuchten *Simplicidentata* eine so lange Epiphyse gefunden. Der Balken überdeckt den Thalamus; die Tubercula superiora der Vierhügelplatte sind über einem stark erweiterten Aquaedukt mächtig enwickelt. Auch im Profil ist die Pars oralis tuberis stark reduziert. Das Corpus mammillare ist sehr gut entwickelt, sowie auch das Chiasma. Die Verhältnisse im medialen Kleinhirnbereich sind in der Abb. 6 schematisch skizziert. Das Kleinhirn ist bei Dolichotis hoch gebaut, vor allem das Culmen-Gebiet ist gut enfaltet. Die Fissura praecentralis, prima und secunda bilden untereinander Winkel von 90°.

Auf den *Grosshirnfrontalschnitten* ist das Rindenband breit, das Marklager ausgedehnt, die Ventrikel eng. Die Stammganglien und der Archicortex sind stark entwickelt.

Familie: Hydrochoeridae
Subfamilie: Hydrochoerinae
Gattung: Hydrochoerus (Brisson, 1762)

Hydrochoerus hydrochaeris (Lin.)

(Abb. 7—8)

Wir konnten nur eine Hirnhälfte untersuchen, der wir die beiliegenden Messungen und Diagramme entnommen haben. Das Hirngewicht beträgt *61,4 gr.* (Messungen von Weber ergeben ein Frischgewicht von *75 gr.*), was dem maximalen Hirngewicht bei Nagetieren entspricht. Das *Verhältnis Hirngewicht (= 1) zum Körpergewicht* wäre nach Weber (♀, 102 cm Körperlänge) *1: 393*, nach alten Angaben von Owen *1: 300*. Die Grosshirnhemisphäre ist im occipitalen Bereich quer abgestumpft und daher annähernd dreieckig. Die Bulbi olfactori sind nur zum kleinen Teil von den Frontalpolen überdeckt. Das Pallium ist besonders dorsal stark gefurcht. Auf der Dorsalfläche finden wir eine Längsfurche, die parasagittal verläuft und ⅔ der Länge einnimmt (Abb. 7 -*a*). Seitlich davon findet sich rostral eine kurze, etwas schräg verlaufende Furche (*b*), die sich in zwei Schenkel, einen medialen (*b'*) und einen lateralen (*b''*) teilt. Im occipitalen Bereich verläuft die caudo-lateral divergierende Furche *c*, die von einer weiteren Furche *d* flankiert wird. Bei der seitlichen und basalen Betrachtung kann man den Verlauf einer *Fissura Sylvii* (*s*) verfolgen, die bogenförmig vom Sulcus orbitalis (*so*) emporsteigt. Caudal davon findet sich die geteilte Furche *e*. Die Fissura rhinalis ist nur lateral vom Pedunculus olfactorius und im Bereich des Lobus pyriformis markiert. Im mittleren Drittel ihres Verlaufes ist sie nur als zarte Spur zu erkennen. Seitlich vom rostralen Segment der Fissura rhinalis verläuft auf der basalen Rinde eine tiefe Furche *so* (Sulcus orbitalis von E. Smith), die nach caudal zieht und in ihrem Verlauf von kleinen querliegenden Furchen unterbrochen wird. Eine davon stellt den Beginn der Fissura Sylvii dar. Im kaudalen Drittel vereinigt sich die Orbitalfurche mit der Fissura rhinalis (*r*) um gemeinsam, zumindest makroskopisch, den Lobus pyriformis abzugrenzen. Im mittleren Bereich des Lobus pyriformis findet sich eine kurze längsverlaufende Fissura intrapyriformis (*i*). Die Medianfläche der Grosshirnhemisphäre ist bei dem von uns untersuchten Präparat beschädigt. Trotzdem ist eine Fissura callosomarginalis um das Knie des Balkens deutlich zu erkennen. Auf der caudo-medialen Fläche der Hemisphäre findet sich eine tiefe Furche; sie beginnt medial vom caudalen Ende der Fissura rhinalis und steigt, ohne mit dieser in Beziehung zu treten, schräg steil empor und erreicht die dorsale Hemisphärenfläche nicht. Ob es sich bei dieser Furche um eine Fissura calcarina (Abb. 7 -x) handelt, wie Beddard vermutet, kann nur der Nachweis einer Area striata an derselben Stelle beweisen. Das Furchenbild des Capybara-gehirnes ist variabel, wie aus dem Vergleich der

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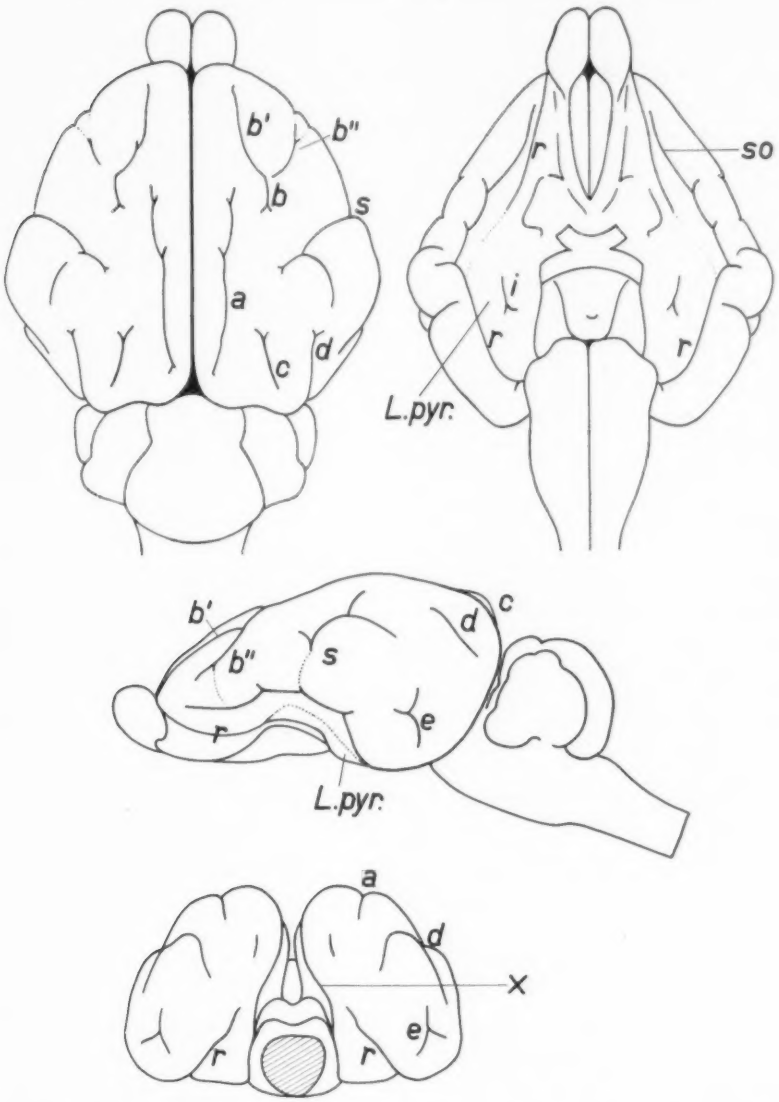

Abb. 7. Gehirn von *Hydrochoerus hydrochaeris,* schematisch gezeichnet: L.pyr. = Lobus pyriformis. Übrige Buchstaben *siehe Text.*

Zeichnungen verschiedener Autoren ersichtlich ist (Abb. 8). Der Balken ist lang und überdeckt den Thalamus. Die Epiphyse scheint beim Capybara auch eine beträchtliche Länge zu haben. Das am Gehirn noch übriggebliebene Fragment ist etwa *10 mm* lang.

Das Kleingehirn ist schmal, der Sulcus paramedianus ist besonders auf der kaudalen Kleingehirnfläche entwickelt. Die Paraflocculi sind breit, und breitflächig den Crura der Hemisphären angewachsen.

Hirnmessungen

Tierangaben	1	2	3
Länge des Grosshirns	44	33	59
Breite des Grosshirns	46	34	64
Höhe des Grosshirns	29	20	33
Länge des Kleinhirns	19	17	22
Breite des Kleinhirns mit Paraflocculi	32	—	38
Breite des Kleinhirns ohne Paraflocculi	23	20	—
Höhe des Kleinhirns m. Brücke	26	16	31
Länge der Brücke	6	4	8,5
Breite der Brücke	16	—	34
Länge des Bulbus olfactorius	13	5	12
Breite des Bulbus olfactorius	8	4	4
Länge des Tractus olfactorius	12	—	23
Breite des Tuberculum olfactorium	7	—	10
Entfernung zwischen den Fissurae rhinales	27	25	40
Fissura rhinalis — Uncus pyriformis	11	10	13
Kleinste Entfernung zwischen den Unci pyriformes	8	6	17
Länge des Hypothalamus	10	6—7 ?	14
Durchmesser des N. opticus	2,5	—	4
	5	—	—
INDEX $\frac{\text{Hypothalamuslänge}}{\text{Grosshirnlänge}}$	0,23	—	0,24
Hypophysenlänge	8	—	—
Hypophysenbreite	9	—	—

1 = Dolichotis patagona (T 1122) — Sammlung *Hirnanatomisches Institut Bern.*
2 = Dolichotis patagona (T 63) — Sammlung *Hirnanatomisches Institut Bern.*
3 = Hydochoerus hydrochaeris IX/24/4 — Sammlung *Max Planck-Institut Giessen.*

Wie aus der morphologischen Beschreibung hervorgeht handelt es sich bei *Dolichotis* und *Hydrochoerus* um zwei für die Nagetiere ungewöhnlich stark differenzierte Gehirne. Dafür sprechen auch der Hypothalamusindex von *0,23* bezw. *0,24,* die bei Cavia zum Beispiel — um eine verwandte unspezialisierte Art zu nennen *0,28–0,30* beträgt. Sowohl die Subfamilie *Dolichotinae* (Familie Caviidae), als auch die Subfamilie *Hydrochoerinae* sind mit einer einzigen Gattung unter den lebenden Nagern vertreten. Bei *Hydrochoerinae* sind vier Gattungen aus dem Pleistocön und Pliocön bekannt, die Gattung *Hydrochoerus* zählt einen einzigen Vertreter, den *Capybara.* Die Subfamilie *Dolichotinae* gruppiert vier pliocöne Gattungen; die heute noch lebende Gattung *Dolichotis* zählt zwei Arten.

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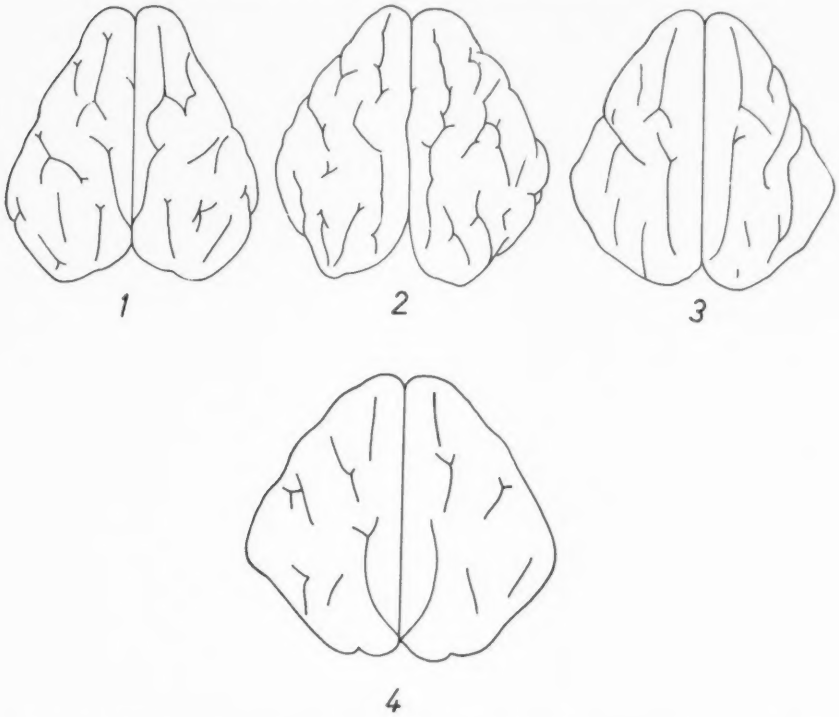

Abb. 8. Variabilität der Grosshirnfurchen bei *Hydrochoerus hydrochaeris:* 1 und 2 nach Beddard, 3 nach Elliot Smith, 4 nach Friant.

Man kann wohl sagen, dass es sich bei beiden Gruppen um phylogenetische Endstadien handelt, sie sind Relikte eines Gattungskreises, der in der geologischen Vergangenheit den Höhepunkt seiner Entwicklung und geographischen Verbreitung erlebt hat. *Hydrochoerus* hat eine ganz eigene Umbildung für eine amphibiotische Lebensweise erfahren: es ist bekannt, dass der Capybara sich mit Leichtigkeit mehrere Minuten lang unter Wasser aufhalten kann (Böckling, zit. von Tullberg). Die Zahl der Zehen beträgt 4 am vorderen, 2 am hinteren Fuss. Die Zehen sind mit kurzen Schwimmhäuten versehen und tragen hufartige Krallen (Tullberg). Die Mara hingegen sind ausgesprochene terrikole Arten, die herdweise in den weiten Pampas und anderen Halbwüsten oder lichten unterholzarmen Buschwaldgebieten von Argentinien und Patagonien leben. Sie sind sehr schnelle Läufer und vorzügliche Springer. Dass es sich bei *Dolichotis* und *Hydrochoerus,* trotz verschiedener Spezialisation, um phyletisch nahe Genera handelt, geht aus der Morphologie der meisten Organe hervor; wie die Meerschweinchen haben sie zum Beispiel wurzellose Molaren, wovon die sehr offenen Spalten mit Zement ausgefüllt sind. Die Gehirne von *Dolichotis* und *Hydrochoerus* sind von

allen Nagetieren am stärksten gyriert. Untereinander sind sie sehr ähnlich und eine Homologisierung der Furchen ist leicht möglich. Das Capybara-Gehirn weist eine etwas stärkere Furchung auf als das von Dolichotis. Was uns von beiden Arten überrascht, ist der gewaltige *Unterschied des endocraniellen Reliefs bei weitgehend morphologisch ähnlich gestalteten Gehirnen.*

Cranio-cerebrale Topographie

(Abb. 9—10)

Dolichotis patagona: im Schädelbinnenraum trennt kein Tentorium osseum das Cavum cerebrale vom Cavum cerebellare. Die Crista petrosa, die scharfkantig von der Flocculusnische steil medial-abwärts verläuft, zeichnet die Grenze zwischen beiden Schädelbereichen. Sie reicht bis zur Spitze der Felsenbeinpyramide, die ungefähr der Mitte der Stelle entspricht. Im Cavum cerebrale fällt sofort die erhebliche Aussparung durch die starke Konvexität des Oribtaldaches auf. Auf diesem steigt vom Foramen lacerum ein mehr oder minder stark ausgeprägtes Jugum osseum zum Schädeldach empor und endet im lateralen Bereich der Sutura fronto-parietalis. Diesem Jugum entspricht am Gehirn der steile Verlauf einer gut markierten Fissura rhinalis posterior, welche die makroskopische palaeo-neocorticale Grenze darstellt. Es kann ausserdem als Grenze zwischen einer vorderen (orbitalen) und mittleren (temporalen) Schädelgrube angesehen werden. In seiner Mitte wird das Jugum durch eine Gefässfurche (A meningica media) leicht unterbrochen. Die endocranielle Fläche des Orbitaldaches ist fast glatt, mit Ausnahme eines kleinen, in der Mitte gelegenen Höckers. Nasal wird das Cavum cerebrale durch ein sehr steil verlaufendes Jugum limitans fossae olfactoriae begrenzt. Die Lamina cribosa ist diesem entsprechend gerichtet. Das Jugum rhinicum zeichnet die scharfe Grenze zwischen Bulbus und Tractus olfactorius und dem frontalen Neocortex. Die Bulbuskammer ist kurz und hoch. Die Fila olfactoria dringen vorwiegend frontal in die Kammer ein. Ein Jugum rhinicum orale ist nur bei einem der beiden von uns untersuchten Schädel als ganz kurze Leiste angedeutet. Von den Formationen der Schädelbasis sind die Foramina optica, entsprechend dem Nervendurchmesser, breit und halbmondförmig. Der Hypophysensattel ist längsoval, etwa 2—3 mm tief. Das Cavum cerebellare ist schmal und hoch. Auf der seitlichen Wand dieses finden sich, durch einen längsgerichteten Knochenbalken getrennt, die Paraflocculuskammer und der Porus acusticus internus. Dorso-caudal ist die Endocranialfläche des Supraoccipitale mit 2 parallel verlaufenden Juga ossea versehen, die dem Sulcus paramedianus des Kleinhirnes entsprechen. Rostral davon finden sich zwei tiefe Sinusimpressionen, die sich lateral an der Crista petrosa verlieren. Auf der endocraniellen Fläche der Schädelkalotte finden sich, entsprechend den parasagittalen Grosshirnfurchen, zwei stark

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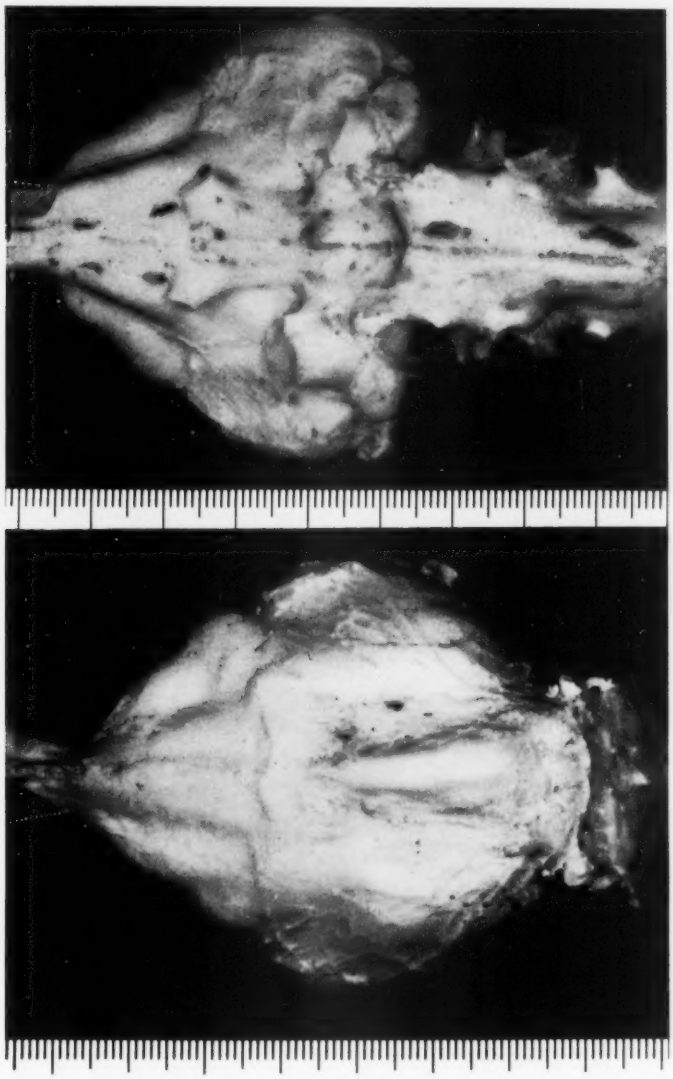

Abb. 9. Endocranialausguss von *Hydrochoerus hydrochaeris,* dorsal und basal gesehen.

ausgebildete gleichfalls parasagittale Knochenkämme, die dem Hirnrelief völlig angepasst sind. Ein dazwischen verlaufendes, etwas schwächeres Jugum, die Ansatzstelle der Falx, enspricht der Sagittalfurche des Grosshirnes.

Hydrochoerus hydrochaeris: die allgemeine Form des craniellen Binnenraumes sind denen von Dolichotis ähnlich. Die Grenze zwischen vorderer und mittlerer

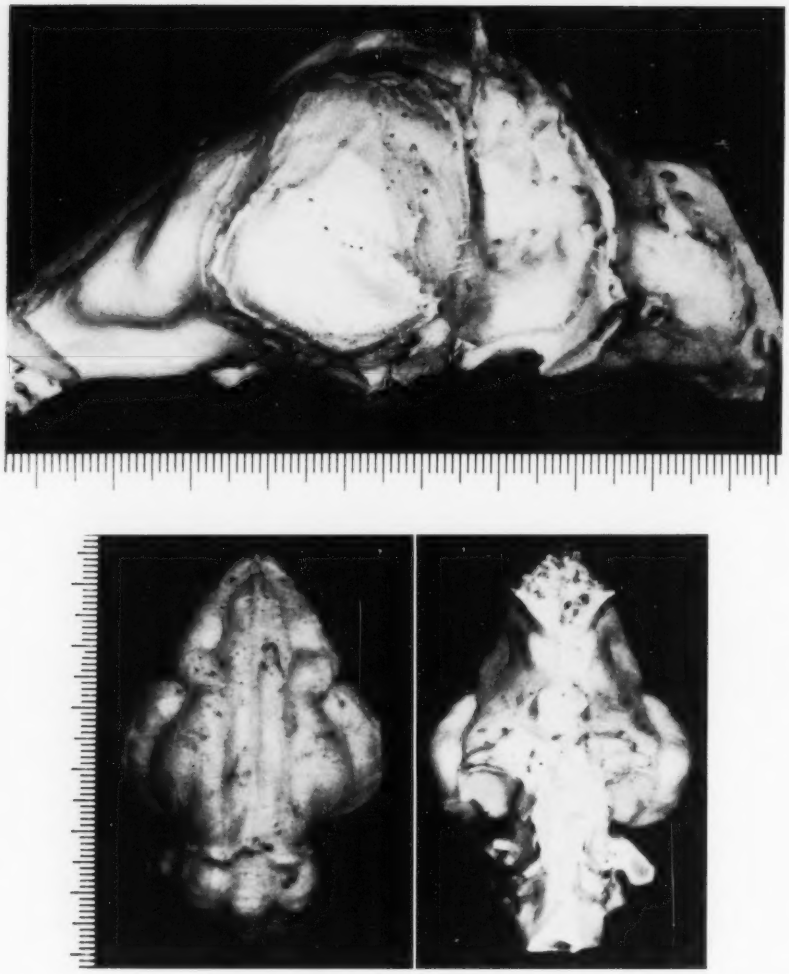

Abb. 10. oben: Endocranialausguss von *Hydrochoerus hydrochaeris,* lateral gesehen; unten: *Dolichotis patagona,* dorsal und basal gesehen.

Schädelgrube ist nur lateral gegeben, im Bereich der Sutura fronto-squamosa, wo die schwache Andeutung eines Jugum sich bemerken lässt. Die nur in ihrer dorsalen Hälfte kantige Crista petrosa des Felsenbeins zeigt den Übergang in die Fossa cerebellaris. Eine mächtige Crista galli halbiert die Bulbuskammer in zwei getrennte Einzelnischen, deren Längsachsen leicht seitlich divergieren. Ein Jugum limitans fossae olfactoriae ist nicht vorhanden, lediglich eine Einengung durch den Knochenvorsprung zeigt den Übergang der Bulbuskammer zur Grosshirnhöhle. Von hier geht ein abgerundetes Jugum rhinicum orale in caudaler Rich-

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tung ab und trifft die Sutura fronto-squamosa. Dem entspricht am Gehirn das Gebiet zwischen Fissura rhinalis anterior und Sulcus orbitalis. Im Bereich der vorderen Schädelgrube erscheint die Endocranialfläche äusserst schwach vom Hirnrelief imprimiert. Das Gleiche können wir von der Innenfläche der Schädelkalotte sagen, wo nur Andeutungen von Impressionen sichtbar sind. Nur das mediane Gebiet erscheint offenbar durch den Verlauf des Sinus sagittalis in Längsrichtung stärker ausgehöhlt. Auch das Gebiet, das dem Chiasma und der Hypophysengegend entspricht, ist reliefarm. Die Sella ist 5 mm tief. Die Fossa cerebellaris ist schmal. Die Paraflocculusnische ist seicht, der Porus acusticus internus ist breit.

Diskussion der Befunde

Wie aus der Beschreibung und den Abbildungen ersichtlich wird, enstpricht zwei sehr ähnlich gebauten Gehirnen, die systematisch verwandten Arten gehören, ein völlig verschieden gestaltetes endocranielles Relief. Während bei *Dolichotis* das Endocranium fast ein Negativum der Grosshirnfurchung darstellt, wird es bei *Hydrochoerus* — der ein noch stärker gyriertes Gehirn aufweist — reliefarm bis glatt. Zur Erklärung einer solchen Tatsache glauben wir in der von SPATZ formulierten, am menschlichen Schädel entwickelten Theorie, einen Weg gefunden zu haben. Das Endocranium wird durch die Rinde „imprimiert". Dies steht wahrscheinlich in Beziehung zur Hirnevolution und zur ontogenetischen Hirnentwicklung. Sind Impressiones an der endocraniellen Fläche vorhanden, beweist das, dass die Rinde ihre Impressionsfähigkeit nicht eingebüsst hat; sie ist, wie SPATZ sagt, *„noch in Entwicklung begriffen"*. „Die Impressionsfähigkeit von Hirnteilen an der Wand des Endocraniums ist ein Indikator für eine diesen Hirnteilen auf der betreffenden Differenzierungsstufe eigene Ausdehnungstendenz" (SPATZ: Propulsivität der Rinde).

Gemäss dieser theoretischen Möglichkeit würden unsere Befunde dafür sprechen, dass das Gehirn des *Hydrochoerus* den Höhepunkt der Differenzierung erreicht, längst überschritten und deswegen seine Propulsivität verloren hat. Das von *Dolichotis* wäre noch differenzierungsfähig oder befände sich gerade auf dem evolutiven Höhepunkt.

Zusammenfassung

Es wird das Gehirn von *Dolichotis patagona* und *Hydrochoerus hydrochaeris* makroskopisch beschrieben und dessen Relief in Beziehung zur Struktur des Endocraniums gesetzt. Der stärker gyrierten Form des Gehirns von *Hydrochoerus* ent-

spricht ein reliefarmes, bis glattes Endocranium, während *Dolichotis* ein ausgeprägtes endocranielles Relief aufweist. In Anlehnung an die Theorie von SPATZ wird diese Tatsache auf die Hirnevolution bezogen.

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The microstructure of skeleton elements in some vertebrates from lower Devonian deposits of the USSR

by

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(Leningrad)

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Fossil vertebrates from Lower Devonian deposits have so far been found on the territory of the USSR comparatively seldom (D. V. Obrutchev, 1938). That is why any new discovery of these fossil vertebrates is of great value.

Some time ago I have received a collection of remains of Lower Devonian vertebrates. These bones of fishes and armour fragments of jawless forms were found on the bank of the creek Krasny, left tributary of the river Nizhni Viluikan (The place of the find: $\varphi = 66° 20' 27''$ N; $\lambda = 107° 01' 15''$ E).

Studying these fragments I discovered two new genera of jawless vertebrates of the order Heterostraci. Small armour elements of a well known Lower Devonian form—*Drepanaspis*—were found in the same pieces of rock. I found a great number of scales of *Porolepsis uralensis* Obrutchev 1938 together with the armour fragments of Lower Devonian Heterostraci as well as a small lower jaw fragment of this Lower Devonian Crossopterygian fish. Two broken teeth were preserved in this fragment.

All this material was so well fossilized that I obtained without difficulty fine transparent sections and could study all the details of microstructure.

I. Sanidaspis sibirica g.n., s.n.

I propose for one of the new genera of Lower Devonian Heterostraci the name *Sanidaspis sibirica* g.n., s.n.

In these collections I found only one small armour fragment of this representa-

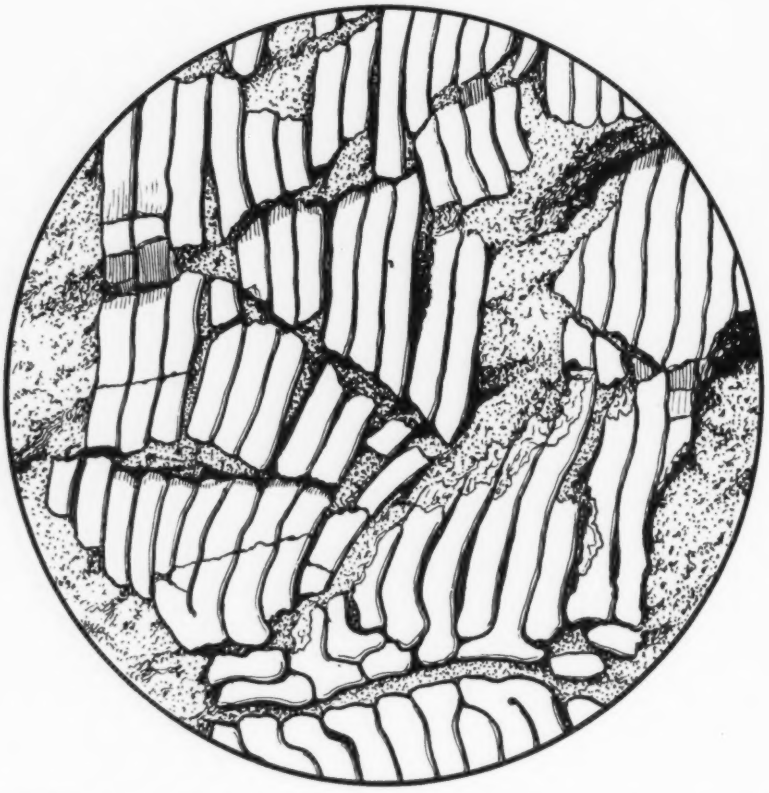

Fig. 1. *Sanidaspis sibirica* g.n., s.n. Fragments of a broken armour in the matrix. × 20.

tive of jawless vertebrates. This fragment was a small part of the shield of the animal and it was impossible to determine its full size. In spite of the insufficient material I have succeeded to prepare both vertical and horizontal transparent sections from small pieces of the armour and to study all the details of the armour microstructure of this new form of Agnatha.

The outer surface of the shield of *Sanidaspis sibirica* g.n., s.n. is covered (at any rate the part which I have at my disposal) with numerous longitudinal glittering plates (fig. 1). These plates have almost the same width throughout and bear a great resemblance to floor boards or deck boards (*σανίς, σανίδος* — a board; I used this word to name the genus *Sanidaspis* g.n.).

The study of the vertical section of the armour of *Sanidaspis sibirica* has shown first of all that the average thickness of its shield is 1,3 mm.

The outer surface of the shield is covered with one layer of dermal teeth (d—fig. 2) Everyone of them is very elongated and has the shape of a plate. Its width varies from 0,30 to 0,35 mm (fig. 1). Everyone of these dental plates

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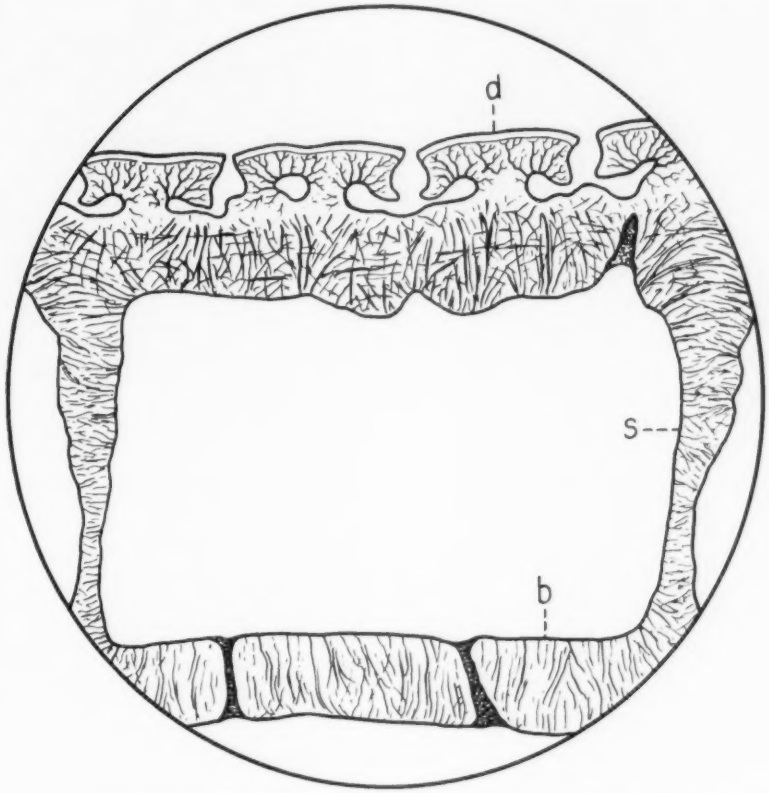

Fig. 2. *Sanidaspis sibirica* g.n., s.n. Vertical section of the armour; b = basal layer; d = cross-section of the tooth lamella; s = vertical septum. × 100.

is connected with the armour by means of a relatively narrow longitudinal trabecula. On both sides of this connecting trabecula two longitudinal pulpar canals pass under every lamellar dermal tooth. Relatively few thin and ramifying dentine tubules branch off from them. These tubules pierce the dentine.

Horizontal canals branch off from the pulpar canals towards the space between neighbouring teeth. These canals are so numerous that vertical sections often cut them longitudinally (fig. 2). In those cases when the section passes between two neighbouring canals one can see the coalescence of the lateral part of lamellar tooth with the armour and the transformation of the pulpar canal into a closed cavity.

The outer surface of lamellar teeth of *Sanidaspis sibirica* is covered with a thick layer of enamel. This layer has a very small longitudinal depression in the middle.

The lateral parts of lamellar teeth were probably covered with a very thin layer

of enamel but even under very high magnification it is impossible to discover this layer.

The upper parts of the spaces between neighbouring lamellar dermal teeth are tapered and have the appearance of very narrow longitudinal slits.

All the lamellar dermal teeth coalesce with the upper layer of the armour. This layer in *Sanidaspis* is as strongly developed as in *Pteraspis* and *Poraspis* and consists of aspidin. Sharpey's canals scattered at irregular intervals are very well seen in it. During the lifetime of the animal they contained collagen fibers (fig. 2).

Large chambers separated from each other by thin vertical aspidin septa (S—fig. 2) are to be found in the middle layer of the armour of *Sanidaspis*. The volume of these chambers in *Sanisdaspis* was greater than in *Tolypelepis, Anglaspis, Poraspis, Corvaspis* (A. P. Bystrow, 1955).

The lower ends of vertical septa are connected with a horizontal basal layer which also consists of aspidin (b—fig. 2).

Canals passing through the layer from one side to another are often seen in it. They served for the passage of the blood vessels carrying blood to the conjunctive tissue which filled up all the cavities of the chambers during the lifetime of the animal.

Horizontal sections of the layer dermal teeth in *Sanidaspis sibirica* show that two pulp canals (fig. 3—upper half) pass under every dentine lamella (d—fig. 3). These canals are connected by numerous horizontal branches with the spaces between neighbouring lamellar teeth. Somewhat more seldom both pulpar canals passing under one dental plate are connected with each other (fig. 3). Studying the arrangement of dentine tubules in dental lamellae of *Sanidaspis* one is unable to find any proof of the fact that these lamellae were formed as a result of the coalescence of several dermal teeth. The formation of dentine crests owing to the coalescence of long rows of dermal teeth can be readily observed studying the microstructure of *Anglaspis* and *Poraspis* (A. P. Bystrow, 1955).

The horizontal sections of lamellar teeth of *Sanidaspis* do not show any enamel since on their lateral surfaces it was either fully absent or very thin. One can see here only a very narrow slit separating from each other neighbouring dental plates (fig. 3—upper half). In horizontal sections of the middle armour layer of *Sanidaspis* it is possible to study only thin aspidin septa forming relatively large chambers (S—fig. 3—lower half).

II. Gunaspis orientalis g.n., s.n.

I propose for the second new genus of jawless vertebrates which was discovered in Lower Devonian deposits in the basin of the river Nizhni Viluikan the name *Gunaspis orientalis* g.n., s.n.

I found very many armour fragments of this animal in my collections. But all

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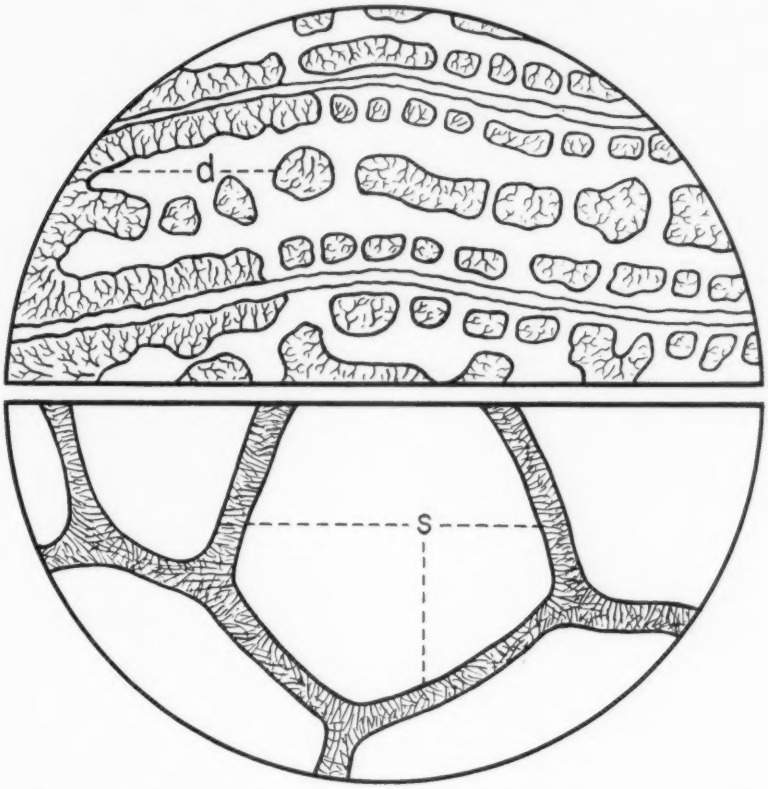

Fig. 3. *Sanidaspis sibirica* g.n., s.n. Upper half—horizontal section of dermal teeth; lower half—horizontal section of the middle layer of the armour; d = dentine; s = aspidin septa. × 100.

of them were fragments firmly connected with the matrix by their external side. Such objects were very convenient for making transparent sections since it was possible to obtain very good preparations in which the external surface of the armour was completely undamaged being protected by the matrix during the making of the sections. All these fragments had one defect: it was impossible to determine the general appearance of relief on the external surface of the armour.

I could study the main details of the appearance of the shield of *Gunaspis orientalis* only because I had at my disposal a small fragment of its armour in which the external surface of the shield was free from the matrix what happens very seldom.

The armour of *Gunaspis orientalis* g.n., s.n. is covered with numerous blunt crests. These crests are arranged in some parts more or less parallel to each other and in others are quite irregularely scattered. They are often curved and ramified.

Fig. 4. *Gunaspis orientalis* g.n., s.n. Outer surface of the armour. × 20.

In some cases they are very long and in others—very short and even have the shape of small round tubercles. All these crests are never quite close to each other (fig. 4). They vary in width from 0,3 to 0,4 mm.

On the armour of *Gunaspis orientalis* among the crests one can notice a very interesting detail: the presence of rather numerous and very unusual large round or oval prominences. Their diameter is usually twice as great as the width of the crests and varies from 0,6 to 0,8 mm. All these prominences have flat tops and their height is always greater than that of the neighbouring crests.

The presence of these unusual flat-topped prominences on the armour suggested to me the name *Gunaspis* for this new genus of Agnatha (*γουνὸς* — hill, elevated platform).

Studiyng the armour of *Gunaspis orientalis* under a magnifying glass one can always easily distinguish a dark spot in the centre of the flat top of every round prominence (fig. 4). The presence of these spots is accounted for by the

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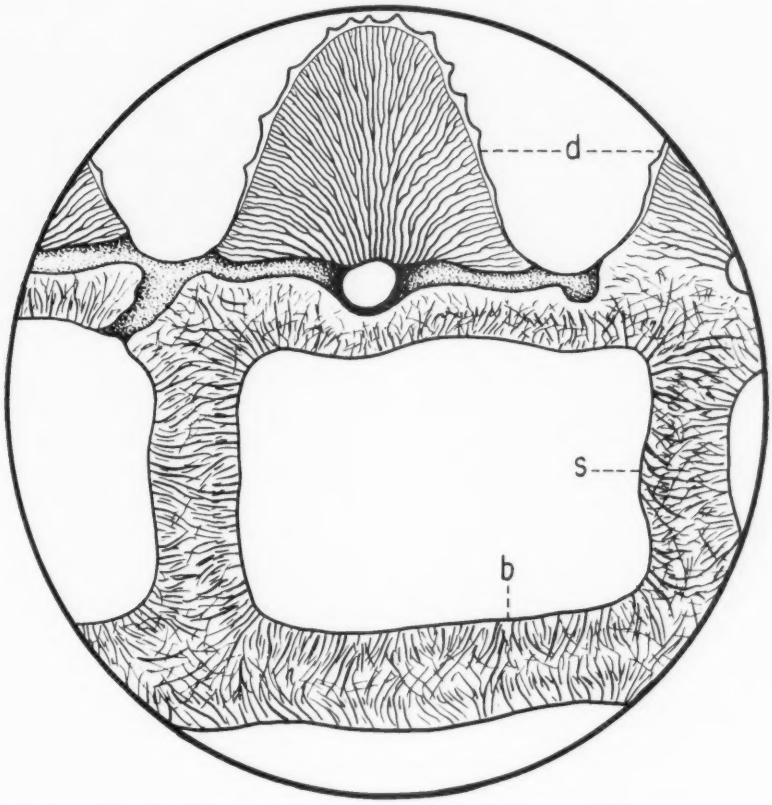

Fig. 5. *Gunaspis orientalis* g.n., s.n. Vertical section of the armour; b = basal layer; d = cross-section of crest-like dermal teeth; s = vertical septum. × 100.

fact that the wall of every prominence is much thinner in the centre portion than on the periphery, being partly ground off. Nevertheless I have never found any holes passing through them from one side to another.

In vertical sections of the armour of *Gunaspis orientated* across the crests it is possible to observe that every crest is a dentine formation (d—fig. 5). Under the crest there is a pulp canal which ramifies into branches opening on the bottom of the depressions between the neighbouring crests. Numerous dentine tubules piercing all the dentine mass of the crest are directed upwards.

All the dentine crests on the armour of *Gunaspis* are covered with a relatively thick enamel layer in which there are 10—13 sharp longitudinal ridges. I have not observed such ridges on the enamel of dermal teeth in any Pteraspidae.

In *Gunaspis* the basis of dentine crests coalesces with the upper lamella of its armour. This lamella consists of aspidin and Sharpey's canals are clearly seen in it.

A series of thick vertical septa forming the walls of spacious chambers (s—

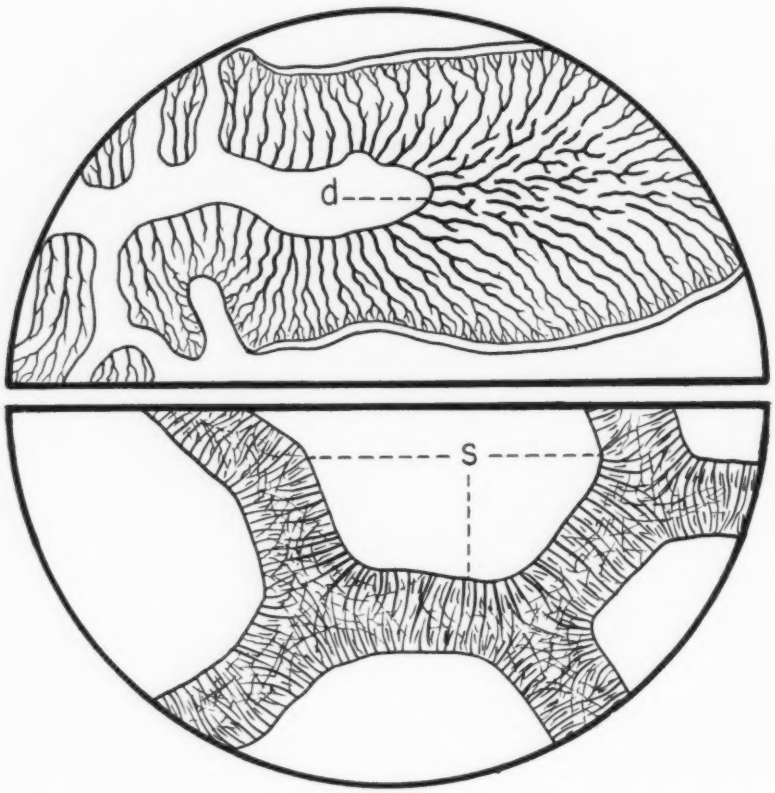

Fig. 6. *Gunaspis orientalis* g.n., s.n. Upper half—horizontal section of the crest-like dermal teeth; lower half—horizontal section of the middle layer of the armour; d = dentine; s = aspidin septa. × 100.

fig. 5) is located in the middle layer of the armour. These septa are connected with basal lamella (b—fig. 5). Both the septa and the basal lamella consist of aspidin. The average thickness of the armour of *Gunaspis orientalis* is 1,5 mm.

The horizontal section passing through the basis of the dentine crest readily shows the pulp canal and its lateral ramifications. In the dentine mass of the crests there are no traces showing that they were formed as a result of the coalescence of a row of dermal teeth (d—fig. 6—upper half) in contrast to that was discovered in the dentine crests on the shields of *Anglaspis* and *Poraspis* (A. P. Bystrow, 1955).

In horizontal sections passing through the middle layer of the armour one can clearly see thick aspidin septa forming the walls of the chambers (s—fig. 6—lower half).

Judging by separate fragments of the armour of *Gunaspis orientalis* the length

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of its dorsal shield was not less than 15 cm. Consequently this representative of Lower Devonian Heterostraci was of a much greater size than Upper Silurian forms.

III. Drepanaspis sp.

Drepanaspis gemündenensis Schlüter discoved for the first time in Lower Devonian deposits of Germany is known to attract the attention of many paleontologists. R. H. Traquair (1903—1905) studied it in greatest detail and made the first reconstruction of this interesting animal. In 1943 the reconstruction of *Drepanaspis* was revised, elaborated and corrected by D. V. Obrutchev. Owing to the existence of a great number of well preserved specimens of *Drepanaspis gemündenensis* it was possible to learn its structure in great detail and at present we have quite a good idea about this Lower Devonian jawless vertebrate.

But no information is available in paleontological literature about the microscopic structure of the armour of *Drepanaspis gemündenensis* except a photo of a transparent section and a schematic drawing of J. Kiær (1915).

J. Kiær wrote in his work that he had succeeded relatively easily in obtaining a good microscopic preparation. But my attempt to repeat Kiær's observations and to prepare a transparent section of a small armour fragment of german *Drepanaspis gemündenensis* did not yield similar results. All the armour of the specimen a small fragment of which was taken by me proved so pyritized that is was quite impossible to investigate its microstructure.

Studying attentively different fragments of scales, bones and the armour of Lower Devonian deposits in the basin of the river Nizhni Viluikan I have found several small hexagonal armour plates. Their maximum width varied from 7 to 10 mm. The external surface of all such plates was covered with tubercles (fig. 7 A). Straight narrow radial grooves and small openings for blood vessels and nerves could be seen on the internal slightly concave side (fig. 7 B). These plates varied in thickness from 0,8 to 1,0 mm.

A great resemblance of the shape of tubercles on the external surface of these polygonal plates with that of the tubercles on the armour elements of *Drepanaspis gemündenensis* Schlüter suggested to me that I had at my disposal the armour plates of just this representative of Agnatha. I think that the small armour plates at my disposal are those numerous polygonal skeleton elements that in *Drepanaspis gemündenensis* are located around its dorsal and ventral shields.

Owing to very good fossilization of armour plates of *Drepanaspis* of Lower Devonian deposits in the basin of the river Nizhni Viluikan I could for the first time obtain fine transparent sections that enable me to get acquainted with the microstructure of this animal's shield using my own preparations.

In vertical sections of the armour plates one can see that every tubercle is a

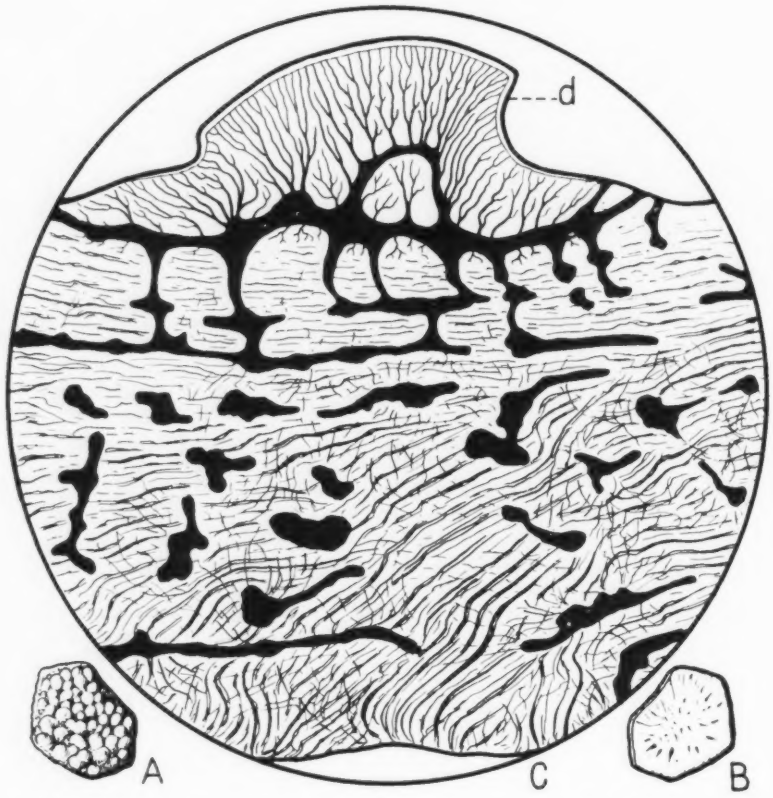

Fig. 7. *Drepanaspis* sp. A = Armour element; view from outside. B = the same armour element: view from inside. C = vertical section of the same armour element; d = dermal tooth. × 100.

small dermal tooth. Usually it has a slightly sharpened inclined top (d—fig. 7 C). All the dentine mass of the tubercle is pierced by numerous ramifying dentine tubules. A system of pulp canals forming extensions in many places is located in the basis of the tubercle.

All dermal teeth of *Drepanaspis* from the basin of the river Nizhni Viluikan are covered with a clearly defined enamel layer. By their general shape they are in no way distinguished from the dermal teeth of *Drepanaspis gemündenensis* Schlüter (J. Kiær, 1915).

The main mass of the armour plates of *Drepanaspis* consists of typical aspidin in which one can well see numerous Scharpey's canals (fig. 7 C).

Irregularly scattered cavities and canals are located in the armour elements of *Drepanaspis*. The middle layer is devoid of those vertical aspidin septa forming

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chambers in the armour shields of many Silurian and some Devonian Heterostraci (*Sanidaspis* and *Gunaspis*).

IV. The Order Heterostraci

The order Heterostraci was defined in 1868 by R. LANKESTER. As we know, Heterostraci have the following characteristics: their head and the front part of the body were covered with shields in which bone cells were absent. The external surface of these shields was covered with dermal teeth unless these teeth were reduced. The branchial apparatus as in all Agnatha was represented by gill-sacs but these sacs have one outer opening on each side. The eyes of Heterostraci were located on the sides of the head and olfactory pits were lying on its front part. Nasohypophysal organ was absent; pineal opening could have been absent. Dorsal, pectoral and abdominal fins were absent. Caudal fin had a hypoceral structure.

I think that we have no reasons to reject the customary name "Heterostraci" through it cannot be considered quite apt and I regard as quite groundless the proposal of L. S. BERG (1940, 1955) to substitute the term Pteraspides for the name Heterostraci and to consider this taxonomic rank a ... class and not an order. In this "class" L. S. BERG distinguished six orders, namely: Astraspiformes, Psammosteiformes, Pteraspiformes, Phialaspiformes, Cyathaspiformes, Amphiaspiformes.

In a well known Zittel's Text-book of paleontology (1932) the order Heterostraci is subdivided into three families, namely: Coelolepidae, Pteraspidae and Drepanaspidae.

The representatives of the family Coelolepidae differed from all the other Heterostraci by the absence of solid armour on the front part of their body. Therefore they should be excluded from the order Heterostraci. Coelolepida should form a separate order as it is done, for instance, by A. S. ROMER (1945).

D. V. OBRUTCHEV (1945) calls Coelolepida "Thelodonti" and considers them a subclass equivalent to Heterostraci which in his opinion form a second subclass of the class Pteraspidimorphi.

It seems to me that we have quite sufficient reasons to exclude Coelolepidae from the order Heterostraci and to subdivide it in only two families, namely Pteraspidae and Drepanaspidae.

The most ancient representatives of the order Heterostraci *Astraspis desiderata* WALCOTT, 1892, *Eriptychius americanus*. WALCOTT, 1892 and *Pycnaspis splendens* ORVIG, 1958 found in Ordovician deposits of the USA had aspidin armour shields on which very unusual dermal teeth were located (W. BRYANT, 1936, T. ORVIG, 1958). These armour elements probably correspond only to the upper layer of the armour of later Heterostraci. Thus *Astraspis, Eriptychius* and *Pycnaspis* are evidently the most primitive representatives of the order Heterostraci and

should be placed in the family Pteraspidae and not in the family Drepanaspidae as was Bryant's opinion (1936).

A careful study of the microscopic structure of the armour shields of Silurian and Devonian representatives of Pteraspidae and Drepanaspidae fully confirms the correctness of introducing these two families.

Family Pteraspidae. The representatives of this family had on the head and on the front part of the body the following armour elements: unpaired rostrale, pineale, dorsale, ventrale, paired orbitalia, branchialia, cornualia. Except these elements there were several plates different form and size behind the oral opening. Some Pteraspidae had sharp spines placed on the posterior edge of dorsal armour and along the back.

The coalescence of some armour elements is observed in different representatives of the family Pteraspidae. Thus, in some forms rostrale, pineale and dorsale coalesced (*Tolypelepis, Anglaspis, Poraspis*), in others—branchiale, and cornuale (*Phialaspis*).

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A very important detail common to all the representatives of the family Pteraspidae without any exception is the presence of vertical septa in the middle layer of their aspidin armour elements. Owing to their presence this layer has a characteristic cancellous structure (fig. 2, 3, 5, 6 of the present work and A. P. Bystrow, 1955,, fig. 11, 12, 14, 15, 17, 18, 20, 21).

On account of the cancellous structure of the middle layer of its armour Pteraspidae possessed maximum strength with minimum use of "building material". Dermal teeth on the outer surface of Pteraspidae's armour had not only a protective function but also mechanical one.

The best known genera of the Pteraspidae family are: *Tolypelepis, Anglaspis, Poraspis, Pteraspis, Protaspis, Corvaspis, Phialaspis, Cyathaspis, Ctenaspis, Dictyonaspis.* Now one must add to them two new genera: *Sanidaspis* g.n. and *Gunaspis* g.n.

Family Drepanaspidae. Drepanaspidae as well as Pteraspidae had aspidin plates on its very flattened head and on the front part of the body. The outer surface of these plates was covered with dermal teeth or else they were fully reduced as in Upper Devonian *Aspidosteus heckeri* Obrutchev, 1941. Many small polygonal armour elements were located around dorsal and ventral shields of Drepanaspidae so that these shields had no direct contact with large aspidinplates forming the edges of the flattened body of the animal (rostrale, orbitale, praebranchiale, branchiale, cornuale). The fields of small polygonal armour elements of Drepanaspidae were placed directly above and beneath the gill-sacs and were a very useful aequisition since they enabled dorsal and ventral shields to move and facilitated the act of breathing. In Pteraspidae the motion of water in the branchial apparatus was probably due exclusively to the contraction of muscles in the walls of gill-sacs. Drepanaspidae had well developed isolated praebranchiale which in Pteraspidae in all probability very early in life fully coalesced with orbitale or with the front part of the branchiale.

The microstructure of Skeleton Elements in some vertebrates

The microstructure of the armour elements of Drepanaspidae is characterized by the absence of cancellous structure of their middle layer. Aspidin septa are disposed in this layer quite irregularly and form a spongy mass not distinguished in any way from the ordinary substantia spongiosa. The armour shields of Drepanaspidae were already sufficiently thick and strong. Probably this accounts for the absence of the regular cancellous structure of their middle layer. Dermal teeth of Drepanaspidae also lost their mechanical function.

Several representatives of the family Drepanaspidae have been found in Devonian deposits. They are the genera *Drepanaspis, Schizosteus, Ganosteus, Pycnosteus, Psammolepis, Psammosteus, Aspidosteus.*

Kallostracon padura LANKESTER (L. Old Red Sandstone, Herefordshire) has the spongy structure of the middle layer of the armour (A. P. BYSTROW, 1955, fig. 22, 23, 24). Therefore this form should also be placed in the family Drepanaspidae and not in the family Pteraspidae as it is done in K. ZITTEL's Text-book of paleontology (1932).

The mysterious form *Cardiopeltis wallacii* BRANSON and MEHL, 1931 from Lower Devonian deposits of North America was at one time considered similar to *Poraspis* and *Pteraspis* on account of the general structure of its armour. This suggested that *Cardiopeltis* should be placed in the family Pteraspidae. But taking into consideration the fact that the middle layer of the armour of *Cardiopeltis* consists of irregularly disposed trabeculae and has no cancellae characteristic for Pteraspidae R. H. DENISON (1953) comes to conclusion that *Cardiopeltis* is much nearer to the family of Drepanaspidae. I think that on account of this detail *Cardiopeltis* should be considered a representative of the family Drepanaspidae.

V. Porolepis uralensis Obrutchev 1938

I have found among the armour fragments of *Sanidaspis sibirica* g.n., s.n. and *Gunaspis orientalis* g.n., s.n. a small fragment of the lower jaw of *Porolepis* which had two broken teeth.

So far as I know, the microscopic structure of the teeth of *Porolepis* has never been studied. Therefore I tried to prepare transparent sections of these teeth.

The long axis of the cross-section of the tooth basis of *Porolepis* in my preparations was 4,0 mm (fig. 8).

The dentine wall of the tooth of *Porolepis* has slight folds resembling the folds in the teeth of *Osteolepis* (A. P. BYSTROW, 1942). The enamel covering the teeth has numerous longitudinal sharp ridges.

In 1935 W. GROSS published the first detailed description of the microscopic structure of the dermal bones of *Porolepis posnaniensis* (KADE). It was found that all the bones of this Crossopterygian fish are covered on the outside with a layer of unusual coalescent dermal teeth very similar in their shape to the teeth

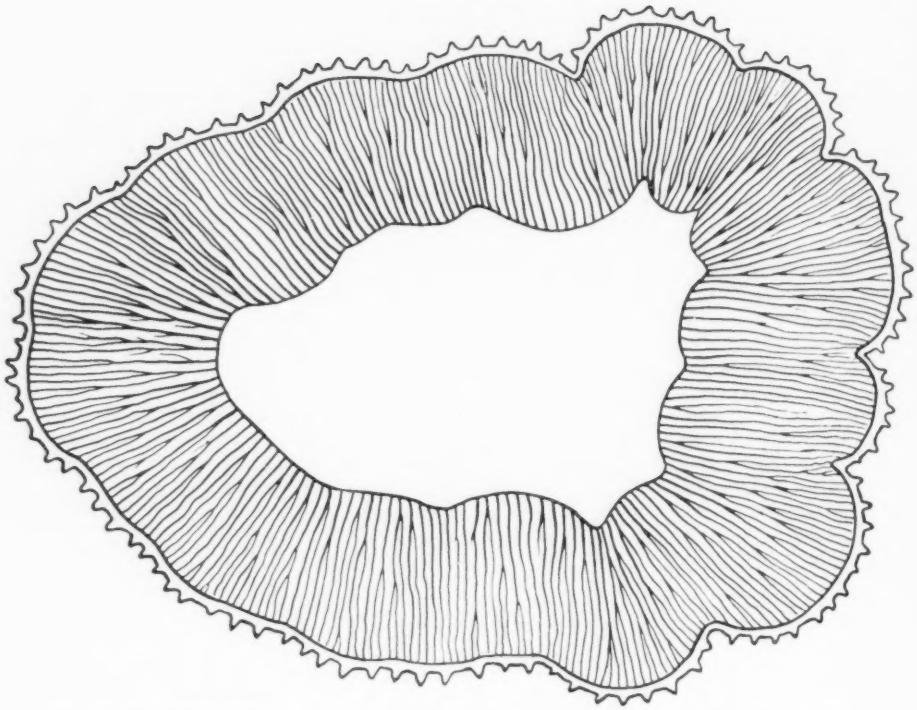

Fig. 8. *Porolepis uralensis* Obr. Cross-section of the tooth. Long axis is 4,0 mm long.

on the bones of *Osteolepis* and *Megalichthys*. I shall call these teeth "parquet dermal teeth" ("Hautzahnparkett", W. Gross, 1930).

A surprising detail of the structure of *Porolepis* discovered for the first time by W. Gross is the presence of a second layer of teeth under the layer of ordinary coalescent dermal teeth. The teeth in this layer have a conical form and are fully immured in the bone tissue. According to W. Gross (1935) they bear a great resemblance to the dermal teeth of *Glyptolepis*.

W. Gross drew from this the conclusion that the scales of young specimen of *Porolepis* at first had been covered with isolated conical dermal teeth which later during the growth of the scale in thickness were immured in the bone. Only after this a layer of coalescent "parquett" dermal teeth appeared on the surface of the scale and these teeth were not replaced during the whole life of the fish.

In his work of 1956 W. Gross again publishes a drawing of the second layer of teeth in *Porolepis posnaniensis* (Kade) and calls them "dermal teeth of the former bone surface" ("Hautzähne der ehemaligen Knochenoberfläche", W. Gross, 1956, p. 68).

During 20 years I could obtain neither the dermal bones nor scales of *Porolepis*

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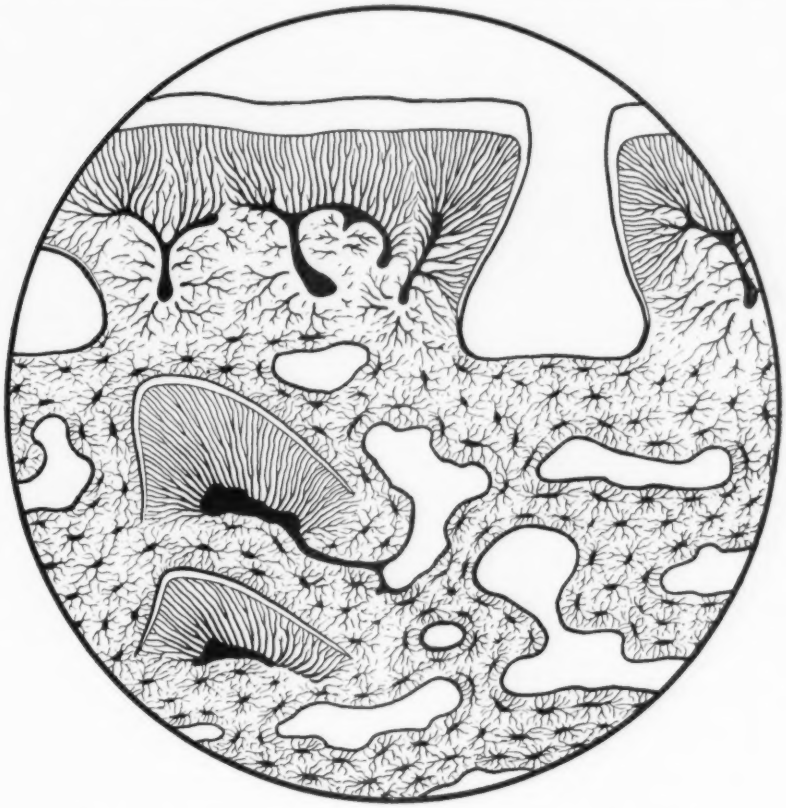

Fig. 9. *Porolepis uralensis* Obr. Vertical section of the scale. × 200.

for microscopic investigations. At present I have at my disposal such material from Siberia and this gives me the possibillity to repeat all W. Gross's observations.

Studying vertical microscopial sections of the scales of *Porolepis* one can see on their surface a well defined layer of coalescent dermal teeth (fig. 9). The height of the teeth in this layer varies with the individual age of the fish but usually is 0,2 mm. Every tooth representing a part of the tooth "parquet" has a flat top and a slightly tapered base coalescent with the bone. The dentine wall of the tooth is very thick and is pierced by a great number of dentine tubules. The pulp cavity is represented by one or several relatively wide canals. The enamel layer on the upper surface of these teeth is very thick and on their lateral parts it is much thinner.

In many places it is possible to find in the bone tissue of the scale under the

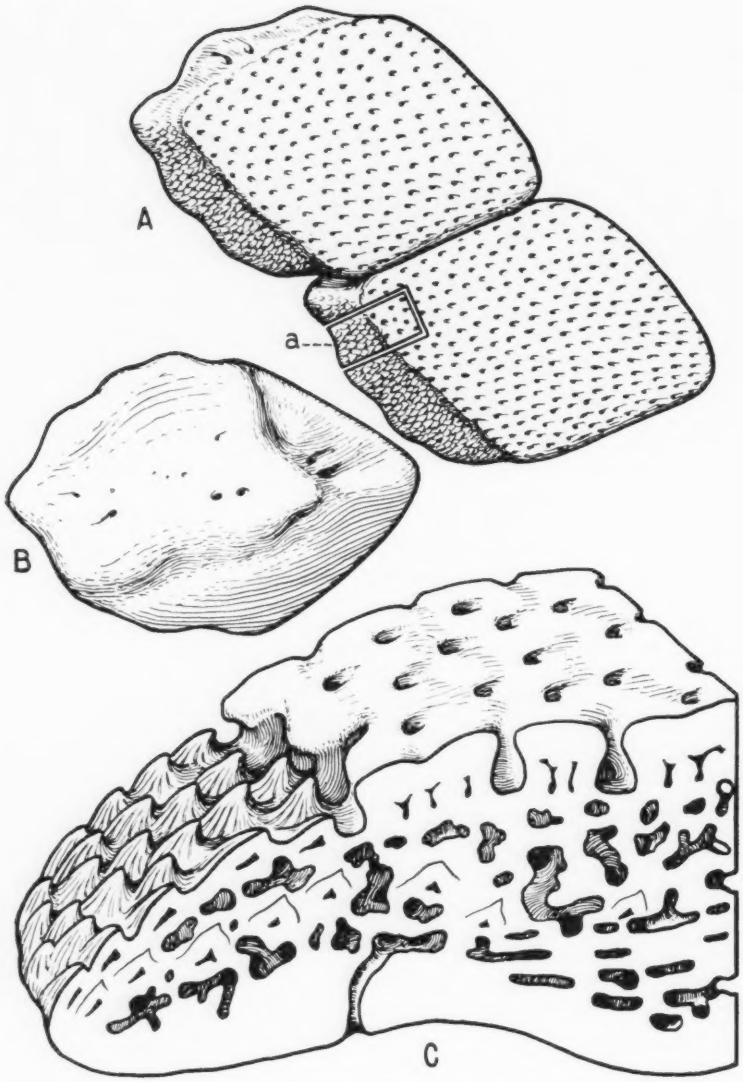

Fig. 10. *Porolepis uralensis* Obr. A = Two scales; view from outside. B = a scale; view from inside. The length of the scales is 10 mm. C = small portion cut out of the front edge of the scale (see a).

layer of "parquet" dermal teeth one or two generations of conical dermal teeth (fig. 9). The shape of these teeth differs greatly from that of the teeth of the "parquet" layer and they never coalesce. Their dentine wall is relatively thick and is pierced by radiating dentine tubules. The pulp cavity usually has the shaps of

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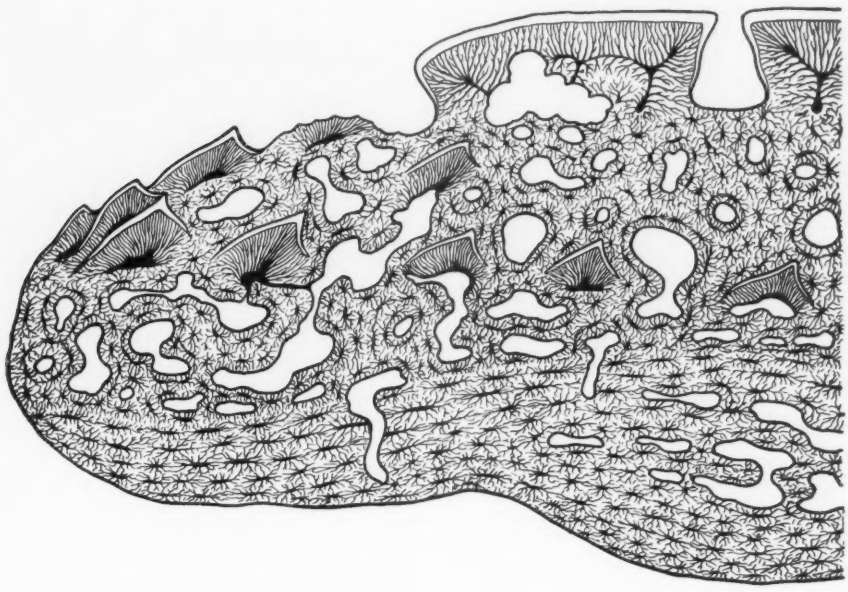

Fig. 11. *Porolepis uralensis* Obr. Vertical section of the front edge of the scale. × 100.

a horizontal slit. The enamel layer usually covers all such tooth and forms a sharp point on its top.

The supposition of W. Gross (1935) that these generations of conical teeth immured in the bone formerly covered all the outer surface of the scale seemed to me both interesting and quite probable. But at present studying the well preserved scales of *Porolepis* I could find some important details that induce me to alter my opinion. The fact is that numerous conical dermal teeth were found on the front parts of the scales of *Porolepis* covered with sharpened back parts of the scales of the preceding row. They are present in greatest number on the frontal-lower slanting edge of the rhomboid scale (fig 10 A). All the surface of this part of the scale is covered with teeth and their sharp tops are always inclined caudalwards. Sharp ridges stretching from the top to the base can be clearly seen on the enamel of these teeth (fig. 10 C).

Studying under the microscope the edges of the scale (a—fig. 10 A; fig. 10 C; fig. 11) one can readily distinguish different generations of conical dermal teeth immured in the bone tissue of the scale. The teeth not yet immured in the bone are located on the slope of this edge. Here they are sometimes literally placed over each other (fig. 11). The sections orientated across the edge of the scale (perpendicularly to the plane of the section represented on fig. 11) and passing through

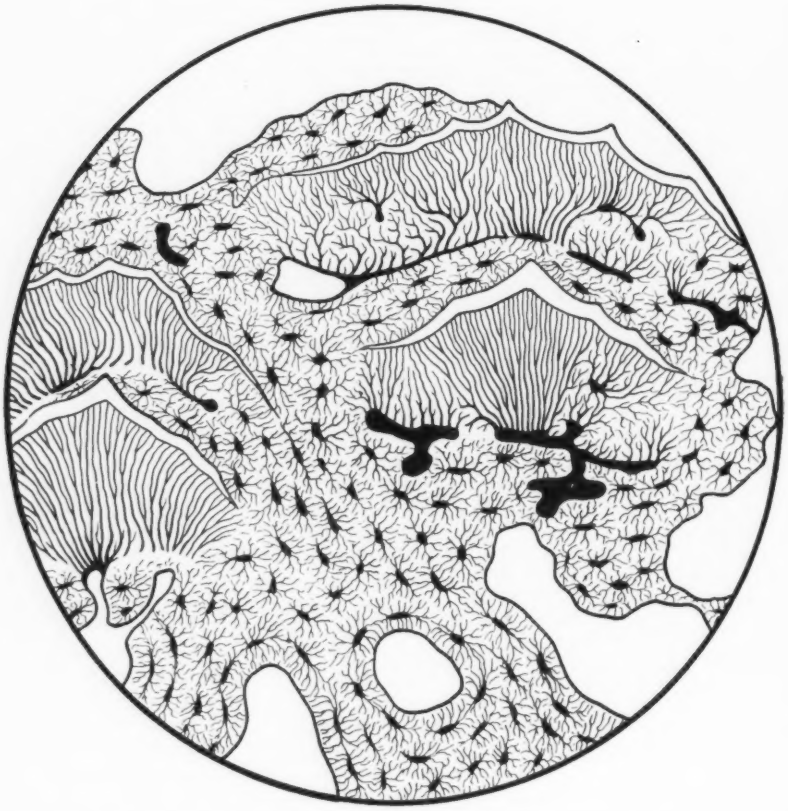

Fig. 12. *Porolepis uralensis* Obr. Vertical section of the front edge of the scale (perpendicular to the surface of the section on fig. 11). × 300.

the zone of conical teeth show the ridge-bearing enamel layer on the surface of those dermal teeth (fig. 12).

The growth of the scales of *Porolepis* evidently took place in the following way: the continuous deposition of new generations of bone tissue on the front edges of the scale caused their gradual extention in the cranial direction. During this process the surface of the slopes of the scale was being covered with conical dermal teeth which were immured in the bone when the edges of the scale thickened. The displacement of the front par of "parquet" teeth in the cranial direction occured simultaneously and this layer gradually covered the immured conical teeth. As a result of all this process different generations of the conical edge teeth were placed in the bone tissue of the scale far from its front edge and sometimes were located deeply under its "parquet" teeth (fig. 9; fig. 11; fig. 13).

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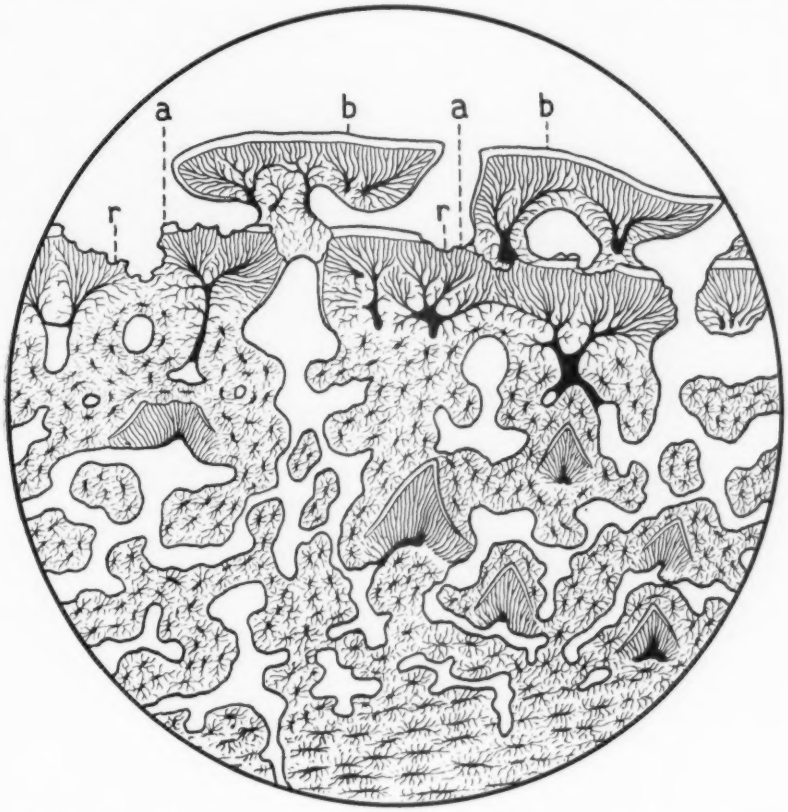

Fig. 13. *Porolepis uralensis* Obr. Vertical section of the scale. a = "parquet" dermal teeth of an earlier generation; b = teeth of the same kind of a later generation; r = resorbed portion. × 100.

Then during the process of the bone rebuilding many conical teeth were destroyded and vanished completely.

In 1942 I published the results of my study of the replacement of consecutive generations of "the parquet of coalescent dermal teeth" ("Hautzahnparquett") in Middle Devonian *Dipterus* and *Osteolepis* (A. P. Bystrow, 1942). I described in this work the destruction of dentine in dermal teeth of consecutive generations. In 1950 E. Jarvik studying the bones of *Dipterus* did not confirm my observations and did not find a single case of destruction of dermal teeth forming a part of the so called teeth "parquet".

E. Jarvik writes that "No spaces of resorption in the pulp cavities of the individual dermal teeth of the dentine layer of the type described by Bystrow (1942) have been observed either along the Westoll-lines or elsewhere" (E. Jarvik, 1950, p.

45). Jarvik evidently did not pay attention to my remark that the phenomena described by me are observed rarely since the replacement of different generations of the "parquet" teeth does not take place continuously (A. P. Bystrow, 1942, pp. 286—287). E. Jarvik evidently hoped to find traces of destruction of "parquet" dermal teeth in every transparent section crossing the boundary between two fields formed by the teeth of different generations. Naturally such hopes could not be justified.

Studying the microscopic structure of the scales of *Porolepis* I could again find the resorption cavities in the "parquet" dermal teeth. The traces of destruction usually could be observed in those elements of dental "parquet" that were located near the front part of the scale (fig. 11). This means that here the "parquet" teeth were replaced first.

I have at my disposal several transparent sections showing that the "parquet" dermal teeth of the first generation semidestroyed by the resorption process are covered with a row of similar teeth of the second generation deposited from above (b—fig. 13). Later the rebuilding of the bone led to full destruction of "parquet" teeth of an early generation. They vanished without leaving any traces.

The "parquet" teeth of a later generation on the front part of the scale not only covered the semidestroyed layer of "parquet" teeth of the preceding generation but also were partly deposited on the conical dermal teeth located in the slope of the scale. As a result of this several layers of immured small conical teeth were covered here with a layer of "parquet" dermal teeth (fig. 13). Evidently R. H. Denison (1951, p. 250, fig. 48) met with the same phenomenon studying the bones of some representative of Osteolepidae.

Thus the growth of the front part of the scale of *Porolepis* was accompanied by a continuous process of immurement of consecutive generations of sharp conical teeth into the bone and of covering them with a layer of "parquet" teeth. During this process the "parquet" of a former generation was substituted by the "parquet" of a new generation. Such substitution of layers of the "parquet" dermal teeth led to the thickening of the scale. Besides, the growth of the scale in thickness also took place on account of the deposition of new bone lamellae on its lower surface. The traces of such deposition can readly be seen on every vertical cross-section of the scale of *Porolepis* (fig. 11).

D. V. Obrutchev in his work "Discovery of Lower Devonian Ichthyofauna in the USSR" writes that "*Porolepis* from the Taimyr peninsula is distinguished from all known species by the presence on the outer surface of the joints of flat tubercles similar to the tubercles on the scales of *Glyptolepis*" (D. V. Obrutchev, 1939, p. 292).

I had at my disposal the scales of *Porolepis uralensis* Obrutchev 1938 from the Taimyr peninsula and from the banks of the river Kureika.

The "tubercles" (= dermal teeth) mentioned by D. V. Obrutchev are present also on the scales *Porolepis* from Lower Devonian deposits in the basin of the

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river Nizhni Viluikan but they cannot be called "flat" (fig. 9, 10, 11, 12, 13). The presence of similar dermal teeth in *Porolepis posnaniensis* (KADE) was discovered by W. GROSS (1935, 1956). I means that these dermal teeth are typical not only for the scales of *Porolepis uralensis* Obr. from the Taimyr peninsula. It is quite possible that the conical dermal teeth on the front part of the scales will be discovered in all species of *Porolepis*. On the scales of the Taimyr specimen of *Porolepis uralensis* Obr. these teeth were "flat" (D. V. OBRUTCHEV, 1939) in all probability only because their sharp tops were broken off. On the preparation of W. GROSS (1935) the tops of the two conical dermal teeth immured in the scale were doubtless destroyed during the rebuilding of the bone.

I have at my disposal no proof of the fact that the outer surface of the scales in young specimens of *Porolepis* and *Osteolepis* was covered with a layer of conical dermal teeth (W. GROSS, 1935). W. GROSS supposes that these teeth were covered with a "parquet" of coalescent dermal teeth only in grown-up fishes. According to W. GROSS the destruction of the teeth and their replacement never took place in this "parquet" of teeth.

My investigations of the microscopic structure of the scales of *Porolepis* have shown that the destruction and formation of new "parquet" dermal teeth can be observed rather often in this Crossapterygian.

As to the conical dermal teeth, they are always present on the front edge of the scale and become surrounded with its bone tissue owing to the intensive growth of its front part. During this process the layer of "parquet" dermal teeth gradually enlarges its area and inevitably covers all the conical dermal teeth immured in bone.

It seems to me that the presence of conical dermal teeth even on the narrow strip of the front edge of the scale of *Porolepis* proves that the scales of the ancestors of Lower Devonian *Porolepis* were covered not with a "parquet" of coalescent specialized dermal teeth but with numerous conical shagreen teeth the sharp tops of which were inclined caudalwards. Nevertheless these teeth did not cover all the outer surface of the scales in young specimens of *Porolepis* or *Osteolepis* as W. GROSS (1935) supposes.

VI. Archaeomycelites odontophagus g.n., s.n.

In 1956 in my work on the destruction of the skeleton elements of fossil animals by fungi I publisher drawings of several preparations in which one could clearly see canals or borings made by hyphae of a saprophyte fungus for which W. Roux had proposed the name *Mycelites ossifragus* (W. ROUX, 1887).

The most ancient form destroyed by the hyphae that I found up to 1956 was a Middle Devonian *Psammolepis paradoxa* Agassiz.

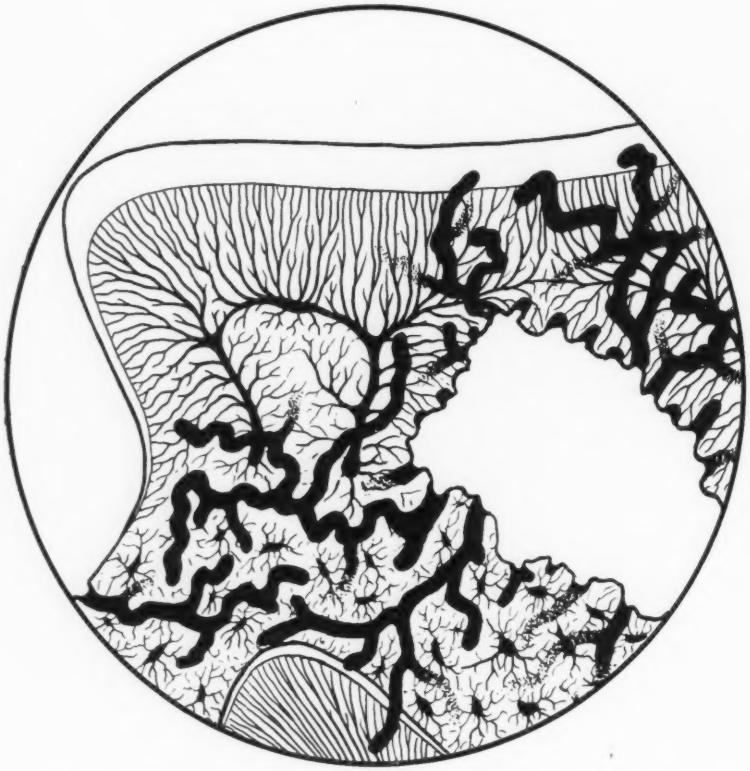

Fig. 14. *Porolepis uralensis* Obr. Vertical section of the scale. One can see the borings made by the hyphae *Archaeomycelites odontophagus* g.n., s.n. × 400.

At present I have also found borings formed by the hyphae of saprophyte fungi in the bones of a Lower Devonian Crossopterygian fish *Porolepis uralensis*, and in the armour elements of a Lower Devonian representative of the family Pteraspidae—*Gunaspis orientalis* g.n., s.n.

The destruction of the bones of *Porolepis uralensis* by the hyphae of a fungus usually started from the space between the two neighbouring "parquet" teeth. During this process the hyphae of a fungus penetrated first through the thin layer of enamel covering the lateral surface of the tooth and then developed in its dentine. Growing through the dentine they in many cases approached from beneath the thick enamel layer lying on the flat upper surface of a "parquet" tooth. I could observe several times the penetration of the ends of the hypha into this layer (fig. 14). Sometime they bored it through and evidently passed outside.

Those hyphae which were directed from the space between the neighbouring "parquet" teeth downwards often penetrated very deeply the bone mass of the

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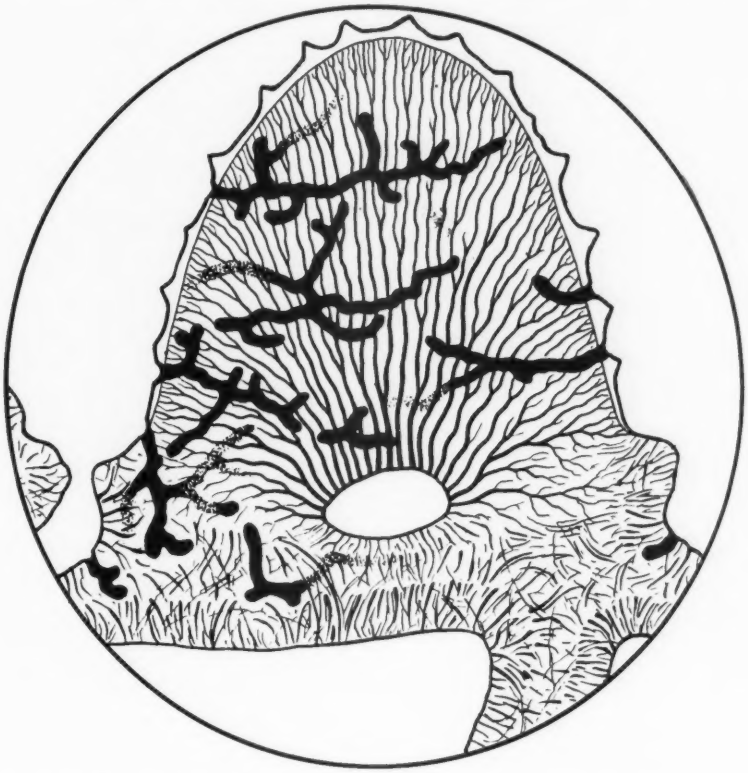

Fig. 15. *Gunaspis orientalis* g.n., s.n. Vertical section of the armour. One can see the borings made by the hyphae of *Archaeomycelites odontophagus* g.n., s.n. × 400.

scale and not only destroyed the bone but also the conical dermal teeth immured in it. Approaching them from above or laterally hyphae usually at first dissolved the thin enamel layer and then penetrated dentine (fig. 14).

Studying a great number of transparent sections of the armour elements of *Gunaspis orientalis* g.n., s.n. I could often find in its crestlike dermal teeth borings made by the hyphae of saprophyte fungi.

They usually penetrated the tooth crest from the outside, evidently easily destroyed the enamel layer and then were introduced into the dentine mass (fig. 15). Those hyphae that penetrated the armour between the tooth crests at once reached aspidin and grew through it (fig. 15).

In *Gunaspis orientalis* the borings made by the hyphae of a fungus can be seen not only in the upper layer of its armour but also in the basal lamella.

The diameter of the borings made by the hyphae of fungi in the bones of *Porolepis* and in the armour shields of *Gunaspis* varies from 5 to 7 μ.

It is well known that the classification of the fungi is based on the study of their spore-bearing organs. Therefore the definition of systematic position of any fungus solely by its hyphae is considered impossible in mycology. Owing to this W. Roux (1887) considered the name proposed by him—*Mycelites ossifragus*—only a working term.

The complete impossibility to discover the spore-bearing organs in ancient saprophyte fungi that left only the borings made by their hyphae in the skeleton elements of fossil animals presents a great difficulty for paleontologists. Taking into consideration all these circumstances we should base our definition of systematic differences in these fungi chiefly on their *physiological* characteristics.

Studying the destruction of the skeleton elements of different fossil vertebrates by saprophyte fungi I (A. P. Bystrow, 1956) found that in marine forms of the cretaceous period the fungi destroyed bone and dentine. I observed no cases of the destruction of enamel. In Upper and Middle Devonian fresh-water vertebrates the hyphae of fungi penetrated bone and aspidin but did not penetrate dentine. This physiological characteristics permitted me to distinguish a saprophyte fungus *Palaeomycelites lacustris* Bystrow 1956 that inhabited the lakes and rivers of Upper and Middle Devonian periods.

R. H. Denison studying the scales of Devonian Osteolepidae complains that "many details of the structure are obscure because of the abundance of borings attributed to fungi (*Mycelites ossifragus*)". (R. H. Denison, 1951, p. 248). Unfortunately R. H. Denison presented neither a description nor a drawing of these borings. Therefore it is impossible to say what was destroyed in the scales of Osteolepidae that he has studied and in what degree.

The investigation of the microstructure of the skeleton elements of Lower Devonian vertebrates (*Porolepis* and *Gunaspis*) has provided me with material proving the presence in fresh-water basins in the beginning of Devon of a saprophyte fungus capable to destroy not only bone and apsidin but also dentine and enamel (fig. 14, 15). Though I have nothing at my disposal except the preparations in which only the borings made by the hyphae can be seen, taking into consideration such important physiological detail of the hyphae as their ability to destroy all hard tissues of Lower Devonian vertebrates (bone, aspidin, dentine and enamel) I think that it is possible to distinguish a new genus of saprophyte fungi. I propose for it the name *Archaeomycelites odontophagus* g.n., s.n. Its genetic name shows the great age of the fungus and the specific name—its ability to destroy teeth.

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A Study of the Nasal Region in a fully formed Chondrocranium of Ovis (Orientalis Gemelin)*

by

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Introduction

A special study of the nasal capsule of a fully developed specimen of Bos Chondrocranium was done by STRUM (1937). DECKER (1883) and WINCZA (1896) studied the primordial skull of the ovis at a particular stage in development. But no attempt has been made to trace the various constituents of the nasal capsule in a sequential manner. An attempt has been made for the first time to study the development of the nasal capsule from the very initial stage and the present description is that of a fully formed chondrocranium.

Method and Material

I have studied the development of the nasal capsule in the following stages.

Stage VI A5—21 m.m. C. R. L.
Stage VII C1—26 m.m. C. R. L.
Stage VIII A7—20 m.m. C. R. L.
Stage IX A1—28 m.m. C. R. L.
Stage X C2—28 m.m. C. R. L.
Stage XI G20—24 m.m. Head Height.

In addition to Van wijhe preparations and serial sections, a wax model of the anterior portion of the nasal capsule has been prepared to show the relationship of the various cartilages.

* Part of the thesis submitted for Ph. D. Degree, Edinburgh University.

Observations

Stage XI G20—24 m.m. Head Height.

At this stage of development, the chondrocranium is fully formed and is in the form of a pear with a narrow anterior end and a broad posterior extremity. The ethmoidal region is bent at an angle to the rest of the cranium and the length of the nasal region is moderately proportional. All the cartilages are fully formed and neither the line of demarkation nor the histological differentiation at the junction of the various constitutes, which have fused together, could be seen. Moreover none of the cartilages have yet begun to ossify.

THE PARS INTERORBITO-NASALIS

A part of this cartilage lies between the optic foramen, and the rest of it continues forward as the nasal septum. Its posterior extent is indicated by an imaginary line drawn through the posterior margins of the metoptic roots of the ala orbitalis. This cartilage is quite thick in the region of the metoptic root, due to the presence of the tuberculum sellae. Anterior to the tuberculum sallae there is a slight depression, the fossa chiasmatica, which lodges the optic chiasma. The fossa chiasmatica is bounded anteriorly again by a rise of the dorsal surface of the central stem which continues forward as a vertical nasal septum. A small portion of this vertical plate lies behind the nasal capsules separating the orbits from one another and the term interorbital septum is applied to this part. The interorbital septum is feebly developed in Ovis as in Bos and Sus. (Fig. 1.)

The Ventral surface is strongly convex from side to side and there is no keel-like projection as is found in reptiles and birds. In mammals the keel has been obscured by the large size of the brain which presses the cartilage from above and the formation of the secondary palate limits the ventral extent of the cartilage.

The nasal septum is the anterior continuation of the interorbital septum, the one passing into the other gradually without any line of demarkation. The nasal septum bears a longitudinal groove on its dorsal border known as the sulcus supraseptalis, which extends the entire length of the nasal capsule in Ovis, as in Homo, Lepus Bos and Sus. The dorsal border of the septum extends backwards in the form of a baek known as the Crista galli process. The ventral border of the septum is convex from side to side and is free for the most part. A little distance from the anterior end it is connected to the lamina transversalis anterior and posteriorly to the lamina orbitonasalis. Between these two connections the inferior border of the nasal septum is in close relationship on each side with paraseptal cartilage and the lamina transversalis posterior, with a narrow septo-paraseptal fissure intervening. There is no perforation of the nasal septum as is found in Sus (Mead 1909).

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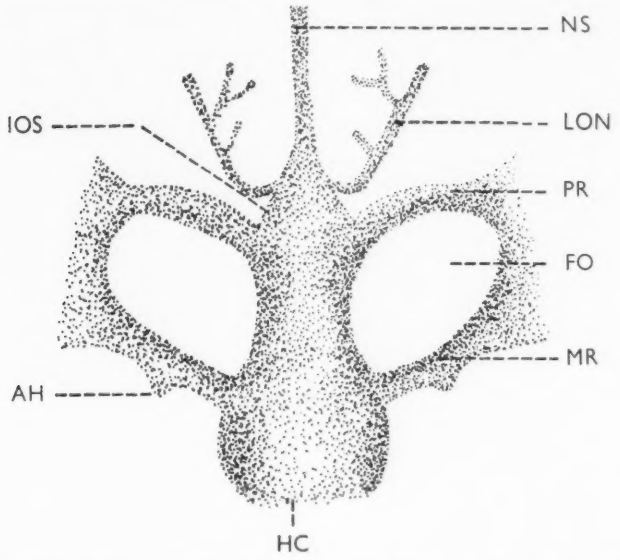

Fig. 1. Inter-orbital septum, situated between Lamina-Orbitonasalis and the Preoptic roots of the Orbital Cartilages.

THE NASAL CAPSULE

Plates LXXIV, LXXV, LXXVII.

The nasal capsule of Ovis is of considerable length and height. It consists of a central stem or the nasal septum, which has been described above and two lateral appendages. The capsule is attached to the rest of the chondrocranium by means of the central stem and by the spheno-ethmoidal commissure at the dorso-lateral angle with the corresponding anterior basal angle of the ala orbitalis. The capsule is bent in such a way that a portion of it comes to lie under the brain and accordingly it may be divided into a subcerebral and a precerebral part.

The nasal capsule is knitted into its final shape and size by three different cartilages, each having separate centres of chondrification, which develop at different intervals. The three cartilages are the parieto-tectal, the paranasal and the lamin-orbitonasalis. The parietotectal cartilage is the first to develop from the dorsal edge of the nasal septum. It forms the roof and anterior side wall of the nasal capsule. The next cartilage to develop is the lamina orbitonasalis which forms the posterior cupola and floor of the nasal capsule. The paranasal cartilage develops next, forming the intermediate portion of the side wall and over-lapping the posterior margin of the parietotectal cartilage and the anterior margin of the lamina orbitonasalis. This results in the projection of the posterior margin of the parietotectal cartilage into the nasal cavity as the crista semicircularis and that of the anterior margin of the lamina orbitonasalis as the first ethmoturbinal.

THE SUBCEREBRAL PART

This part forms nearly a quarter of the entire length of the nasal capsule. It consists of a pair of cartilaginous plates, the laminae cribrosae separated by means of the nasal septum. Each lamina cribrosa is in the form of a triangle with the base towards the nasal septum. The anterior arm of the triangle is short and forms the posterior boundary of the roof of the nasal capsule. This border extends from the root of the crista galli, the backward extension of the nasal septum, to the postero-lateral angle, where it joins the spheno-ethmoidal commissure. The postero-lateral arm of the triangle is longer than the anterior and forms the anterior boundary of the orbitonasal fissure.

There are four ridges emerging from the postero-lateral wall, the crista intercribrosa which runs obliquely from behind forward and inwards across the lamina cribrosa, dividing it into a number of compartments. Two of these intercribrosa are of considerable size and may be regarded as primary ridges when compared with the smaller ridges, the secondary crista intercribrosa situated on either side of them. These ridges divide the cribrous plate into a number of compartments which are further subdivided into a number of smaller foramina through which the olfactory nerves pass. The laminae cribrosae are pushed from their primitive vertical position into an almost horizontal plane by the greater expansion of the brain in mammals in general. The position of the cribriform plates of Ovis prove no exception to this.

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THE PRECEREBRAL PART

The roof of the precerebral part of the nasal capsule is formed by the parietotectal cartilage, which descends gradually forwards from the lamina cribrosa. The parietotectal cartilage is attached to the dorsal border of the nasal septum in such a way that a longitudinal groove, the sulcus suproseptalis runs all along the length of the nasal capsule between the two cartilages. The junction of the parietotectal cartilage with the paranasal is marked by the presence of a small formen, the epiphanial foramen, through which the nasal branch of the profundus nerve passes. Below this foramen, the junction of the two cartilages is marked by a sulcus which runs vertically downwards and forwards; the sulcus lateralis anterior. This sulcus coincides in the interior with the attachment of the crista semicircularis. The anterior border of the tectum nasi forms the lateral boundary of the fenestra narina. (Fig. 2.)

For the convenience of description the lateral surface of the capsule may be divided into three parts namely, the pars anterior, pars intermedia and pars posterior. These parts are formed by the parietotectal, paranasal and the lamina orbitonasal cartilages respectively.

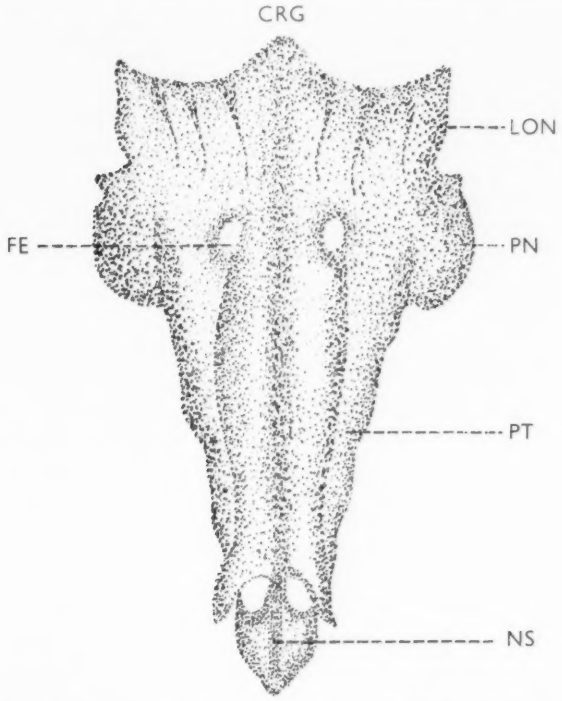

Fig. 2. Dorsal view of the entire nasal capsule, showing the Crista Galli process, Epiphanial Foramen, and the Sulcus Supraseptalis.

THE PARS POSTERIOR

This portion is formed as mentioned above by the lamina orbitonasalis and is the smallest of the three segments. It is separated from the pars intermedia by means of a sulcus, the sulcus lateralis posterior, which runs vertically downwards and backwards, behind the bulging portion of the paranasalis till it reaches the ventral margin of the cartilage. The posterior sulcus coincides in the interior with the line of attachment of the first ethmoturbinal.

The lateral surfaces of the cartilage gives an appearance of a quadrilateral plate turned inside at the postero-ventral end forming the posterior cupola. The posterior margin of the cartilage forms the anterior margin of the orbitonasal fissure. The anterior margin is attached to the paranasal cartilage at the sulcus lateralis posterior. The postero dorsal angle is continued backwards as the sphenoethmoidal commissure. Postero-ventrally the cartilage turns inside forming a considerable portion of the floor of the posterior cupola. It continues further forward on the floor to form the lamina transversalis posterior, which is separated from the ventral margin of the nasal septum by means of a fissure.

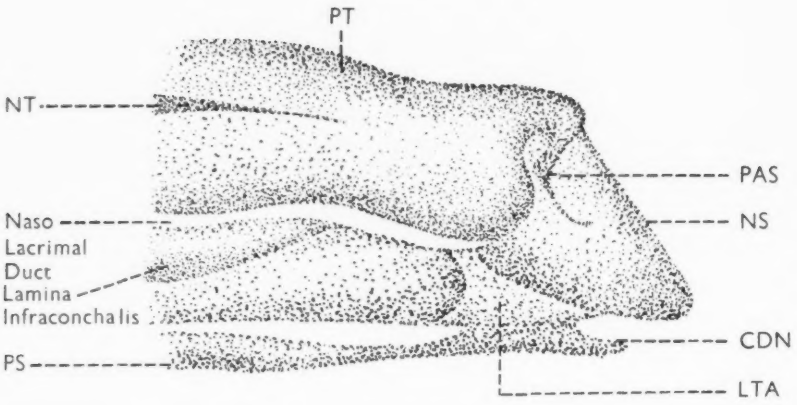

Fig. 3. Lateral view of the Anterior region of the Nasal Capsule, showing the Processus Alaris Superior and the Lamina Transversalis Anterior, forming the Zona Annularis. The Paraseptal cartilage is seen extended forward, as the Cartilago Ductus Naso-palatini.

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THE PARS INTERMEDIA

The paranasalis constitutes this part. The cartilage bulges out externally in the form of a globe beyond the general contour of the lateral surface of the nasal capsule. It is marked off anteriorly from the parietotectal cartilage by means of the sulcus lateralis anterior and posteriorly from the lamina orbitonasalis by the sulcus lateralis posterior. Close examination of the lateral surface of this cartilage reveals, besides the main ventral bulging, two more smaller prominences, one at the posterodorsal angle; the prominentia superior or frontalis, and another at the anterior margin; the prominentia inferior or maxillaris. These prominences correspond with the hollows in the interior of the capsule, thus the prominentia frontalis corresponds with the recessus frontalis, and the prominentia inferior with the recessus maxillaris.

The ventral margin of the paranasalis rolls inwards and forms the floor of the recessus maxillaris. It is continuous posteriorly with the lamina transversalis posterior and with the ventral margin of the parietotectal cartilage anteriorly. The epiphanial foramen is situated at the dorsal third of the cartilage.

THE PARS ANTERIOR

This part is formed by the parietotectal cartilage. It is the longest segment of the nasal capsule. The posterior border of the cartilage is over lapped by the paranasal cartilage and projects into the nasal cavity as the crista semicircularis. This is marked externally by the sulcus lateralis anterior. The posterior part of the parietotectal cartilage bears inferiorly, a longitudinal groove beyond which the cartilage extends downwards below the level of the maxillo turbinal. This

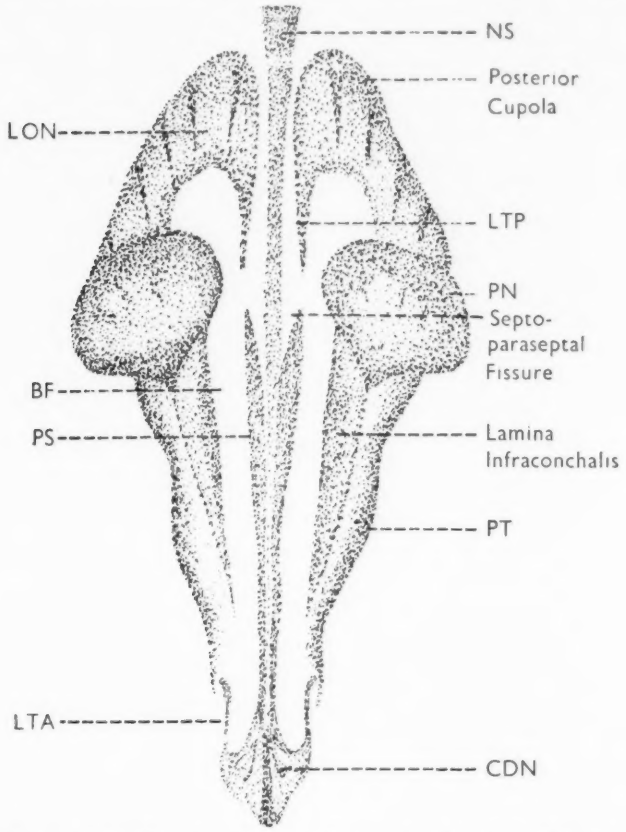

Fig. 4. Boundaries of the Fenestra Basalis and the extension of the Paraseptal Cartilage, on either side of the nasal septum, with the Septo-Paraseptal fissure in between.

portion of the cartilage is termed the lamina infroconchalis from its position. The nasolacrimal duct runs in the groove and hence the term; the nasolacrimal sulcus. The cartilage is joined anteriorly to the lamina transversalis anterior, which connects it to the nasal septum. There is no fissure between the nasal septum and the lamina transversalis anterior, hence a complete ring is formed, the zona annularis. The anterior free border of the parietotectal cartilage ascends upwards to end in a notch formed by the downwardly directed process, the processus alaris superior. There is no cupola anterior in Ovis as the fenestra narina opens directly in front. (Fig. 3).

THE FLOOR OF THE NASAL CAPSULE

The nasal capsule is closed on all sides except the anterior and the ventral. The anterior opening is the fenestra narina. Behind the lamina transversalis anterior

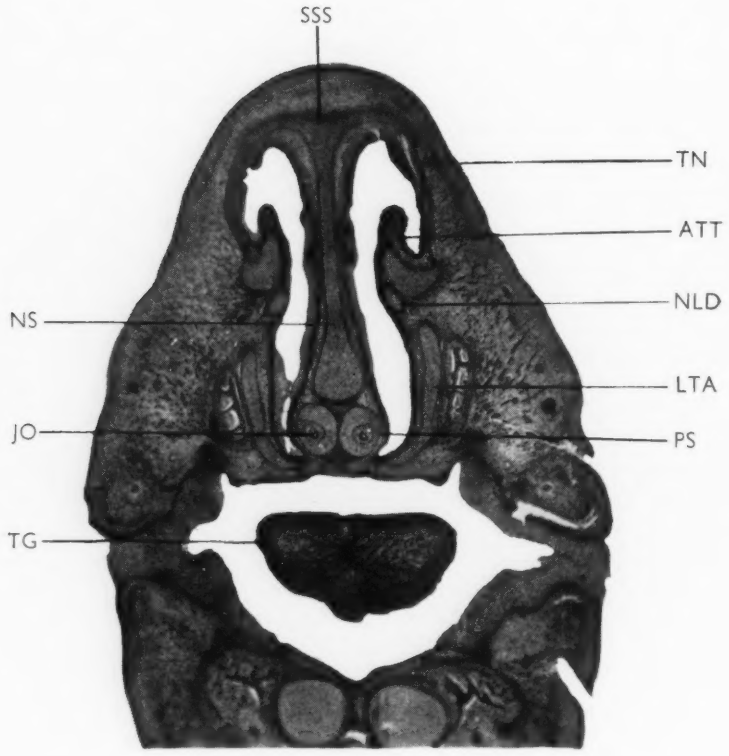

Fig. 5. Tube-like formation of the Paraseptal Cartilage and the posterior part of the Lamina Transversalis Anterior.

is one of the ventral vacuaties; the fenestra basalis. The fenestra basalis is surrounded by cartilages on all sides, namely, anteriorly by the lamina transversalis anterior, posteriorly by the lamina transversalis posterior, medially by the paraseptal cartilage and laterally by the ventral margin of the paries nasi. The aperture of the fenestra basalis is narrowed by the presence of the cartilago-ectochonolis extending backwards from the lamina transversalis anterior. The second vacuity is the septo paraseptal fissure, which intervenes between the paraseptal cartilages and the lamina transversalis posterior laterally, and the infero lateral border of the septum nasi medially.

The paraseptal cartilages is a paired structure extending beside the ventral edge of the nasal septum. It encloses the Jacobson's organ in its anterior portion. Describing the paraseptal cartilage from behind forwards, it is in the form of a solid cartilaginous rod posteriorly. It continues forward for a little distance as a cartilaginous plate (medial lamella) by the side of the ventral margin of the nasal

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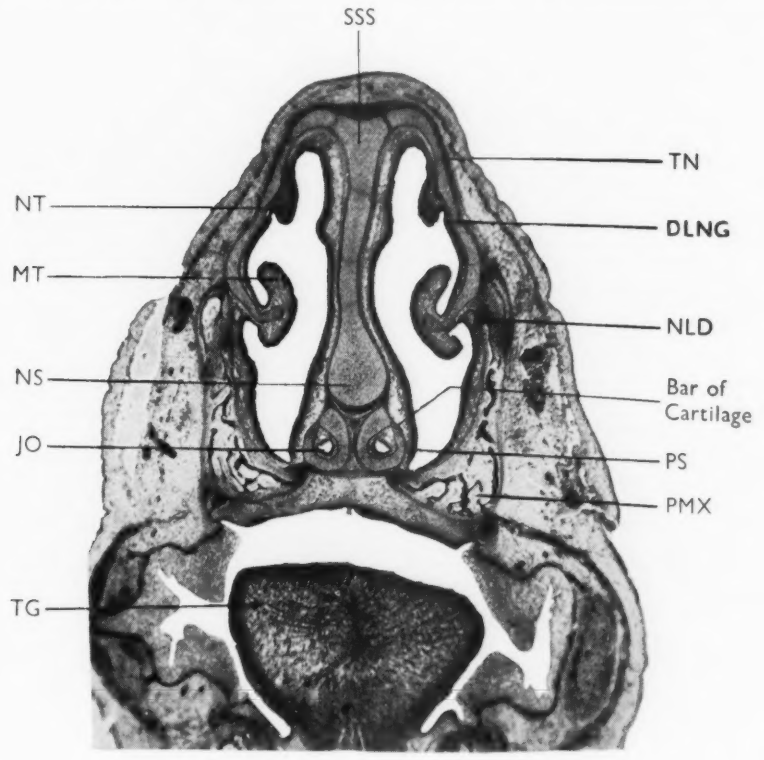

Fig. 6. Outer bar of cartilage attached to the lower limb of the "C" shaped Paraseptal Cartilage.

septum, which soon assumes an L shaped form. The two arms of the L shaped cartilage become equal in length as traced forward with the result it may be mentioned that the paraseptal cartilage in this region is bilaminar with a medial and a lateral lamellae. Soon these two lamellae meet together to form a tube in which Jacobson's organ is lodged. The para septal cartilage is continued further anterior to the lamina transversalis anterior, as the cartilago ductus nasopalatini with a ventral longitudinal opening. (Fig. IV.)

THE MEDIAL ASPECT OF THE LATERAL WALL OF THE NASAL CAPSULE

The medial aspect like the lateral is divisible into the posterior, the intermediate and the anterior parts.

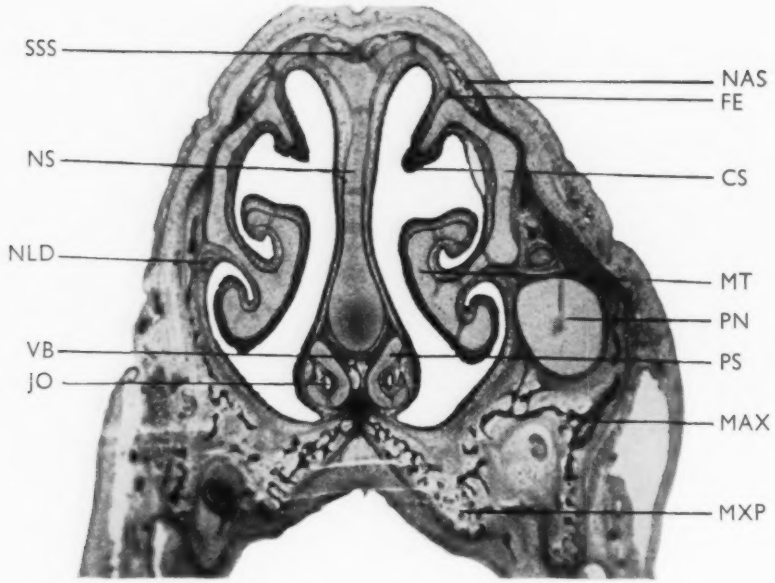

Fig. 7. The "L" shaped nature of the Paraseptal Cartilage in the posterior region. Crista Semicircularis and the gap above it, the Epiphanial Foramen are clearly indicated.

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THE PARS POSTERIOR OR PARS ETHMOIDALIS

This is characterised by the presence of a number of ethmoturbinals projecting into the nasal cavity from the lateral wall and the lamina cribrosa. There are three major or primary ethmoturbinals and are named from before backwards, the first, second and third according to the priority of their development. The first ethmoturbinal coincides on the external surface of the nasal capsule with the sulcus lateralis posterior. It is the biggest and is characterised by three roots, which are continuous with one another. The first turbinal has two lamellae, the dorsal and ventral. The dorsal lamellae, is the larger of the two and projects anteriorly over the anterior root, thus hiding from view, from the medial aspect, a considerable portion of the pars intermedia.

The ventral lamella is shorter than the dorsal and is attached only by the ventral root in common with the dorsal. Between these two lamellae could be seen the secondary ethmoturbinals.

The second primary ethmoturbinal is smaller than the first and is also characterised by the presence of two lamellae. The third primary ethmoturbinal is the smallets of the three being situated at the ventral part of the pars posterior. It extends from the lamina cribrosa forwards to end on the medial aspect of the

lateral wall of the nasal capsule. The secondary ethmoturbinals are situated in the hollow of the second and third primary ethmoturbinals.

THE PARS INTERMEDIA

This part appears on the external surface like a dome. It is limited posteriorly by the first ethmoturbinal and anteriorly by the crista semicircularis. A considerable portion of the pars intermedia is hidden from view on the medial aspect by the forward extension of the anterior root of the first ethmoturbinal. This root divides the deeply concave recess of the pars intermedia into a superior or frontalis recessus and an inferior or recessus maxillaris. In front of the anterior root of the first ethmoturbinal, these two recesses are continuous by means of a narrow recess, the recessus anterior. The ventral margin rolls in wards forming the backward extension of the maxillo-turbinal. Anterior to the recessus maxillaris and inferior to the crista semicircularis is the recessus glandularis in which the lateral nasal glands are lodged. The recessus glandularis is not clearly perceptible as it is the smallest recess without any boundary line and is occupied by the lobes of the gland.

THE PARS ANTERIOR

This part is like a scroll attached to the nasal septum, triangular in shape with the apex anteriorly. The anterior margin forms the lateral boundary of the fenestra narina. The pars anterior is divided in its length into two unequal portions by the nasoturbinal which stretches longitudinally. The inrolling of the ventral border of the parietotectal cartilage forms the atrioturbinal. It is continued posteriorly with the maxillo-turbinal without any interruption.

Discussion

The part of the central stem posterior to the nasal capsule and anterior to the preoptic roots of the ala orbitalis is the interorbital septum. The presence of a keel like and well developed interorbital septum is a primitive character found in reptiles and birds. It is associated with the large size of the eyeballs, which press the interorbital septum into a thin vertical plate between the eyes and push the brain case to the more posterior region of the skull. In mammals, the vertical appearance is obscured by the greater development of the brain which extends forwards and compresses the central stem downwards and at the same time by the formation of the secondary palate. The presence of a well developed interorbital septum in mammals indicates a primitive character exhibited by the reptilian ancestors.

On account of the backward extension of the lamina orbito-nasalis, a portion of the original interorbital septum has been usurped into the nasal cavity as part of the nasal septum resulting in some mammals having a feebly developed inter orbital septum, e.g. Canis (Olmstead 1911), Bos (Fawcett 1918 A), Otomys (Eloff 1951) and Ovis. In primates, Homo (Macklin 1921), Semnopithecus (Fischer 1903), Macacus (Fischer 1903), Chrybothrix (Henkel 1928 A) and in Rodents, Lepus (Voit 1909), and Microtus (Fawcett 1917), there is a well developed interorbital septum. This may be connected with the fact that in Primates the nasal capsule is reduced in size with the result that a greater length of the nasal septum is left behind the nasal capsule as the interorbital septum.

The degree of extension of the nasal capsule is indicated by the length and fussion of the paraseptal cartilage with the lamina transversalis anterior and posterior. Rodents show the paraseptal cartilage still attached to both the anterior and posterior lamina transversalis, thus indicating that the backward extension of the nasal capsule does not break off the union between the paraseptal cartilage and the lamina transversalis posterior. Hence a well developed interorbital septum is observed in Rodents. In other mammals like Ovis the paraseptal cartilage is seen stopping short of the lamina transversalis posterior, thus indicating a backward extension of the nasal capsule which has incorporated a portion of the interorbital septum into the nasal cavity as the nasal septum, and so a feebly developed interorbital septum is present.

The first cartilage to develop in the nasal capsule is the parietotectal from the dorsal edge of the nasal septum. It lodges a longitudinal groove; the sulcus supra septalis which resembles those of Homo, Lepus (de Beer 1930), Bos (Strum 1937) and Sus (Mead 1909).

The lamina orbito-nasalis and the paranasal cartilages develop next. The remanent of the original gap betwen the two cartilages before the fusion is represented by the epiphanial foramen through which the nasal branch of the trigeminal nerve passes. It is interesting to note that there is no fenestra septi in Ovis. Sus (Mead 1909 and Parker 1874), shows a thin portion in the anterior part of the nasal septum, a short distance from the nasal opening. In Talpa (Fischer) there is one opening at this place filled with connective tissue. The presence of fenestra septi is considered as an adoptation in connection with the flexibility of the nose (Spurgat 1896).

Another interesting feature in the nasal capsule of Ovis is the absence of the cupola anterior since the narinal opening is directed forwards and also the absence of the fenestra superior nasi on the lateral wall. The joining of the lamina transversalis anterior with the nasal septum gives rise to a complete ring of cartilage, the zona annularis. Similar is the case in Microtus (Fawcett 1917), Sorex (de Beer 1929), Erethizon (Struthers 1927) and Canis (Olmstead 1911).

The lamina transversalis posterior does not fuse with the nasal septum and as such a narrow fissure, the septoparaseptal fissure is found as in Lepus (de Beer

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and Woodger 1930), Microtus (Fawcett 1917), Sorex (de Beer 1929) and Felis (Terry 1917). The paraseptal cartilages do not fuse with the lamina transversalis posterior, which explains the fact that the nasal capsule has been extended backwards resulting in the breaking of union between the two. The same argument stands to justify the feeble development of the interorbital septum since the nasal capsule has extended backwards incorporating a part of the original long interorbital septum into the nasal cavity.

Broom in his series of articles on the nasal capsule of different mammals, traced that part of the paraseptal cartilage containing the Jacobson's organ. He classified the mammals into two groups, namely, 'Archaeorhinata' containing an outer bar of Jacobson's cartilage and 'Caenarhinata' including Artiodactyla, Perisoodactyla, Carnivora and other groups in which the outer bar is absent and the cartilago ductus nasopalatini is present. The present investigation confirms that Ovis belongs to the Caenarhinata groups on account of the presence of a distinct cartilago ductus nasopalatini, as do equus (Spurgat 1896) and Bos (Michalkovics 1899). At the same time the outer bar is also present, in the form of a short rod attached to the lower limb of the 'C' shaped paraseptal cartilage. Hence it may be concluded that though a distinction has been drawn between the two groups it is perhaps bridged over by forms possessing the character of both groups. A distinct outer bar has been recognised in Sus, Equus and Sorex though they belong to the Caenarhinata group.

The palatine cartilage which represents the primitive cartilaginous nasal floor is also found in Ovis as in many other mammals, Echidna (Parker 1894, Gaupp 1908), Sorex (de Beer), Tupaja (Broom 1915), Lepus (Broom 1900, Voit 1909) and Felis (Broom 1900, Terry 1917).

Summary

1. There is a short orbitonasal septum between the preoptic root of the ala orbitalis and the lamina orbito-nasalis.
2. The nasal septum projects backwards dorsally in the form of a beak, the crista galli process.
3. There is no fenestra septalis in Ovis.
4. The cribriform plate assumes a horizontal position from its primitive vertical position.
5. There is a sulcus supra-septalis dorsally, extending the entire length of the nasal septum.
6. The sphenethmoid commissure is in the form of a thin rod extending from the postero-dorsal angle of the nasal capsule, arching over the orbitonasal fissure.

7. There is no fenestra superior nasi.
8. There is septo-paraseptal fissure between the nasal septum and the paraseptal cartilages.
9. There is zona annularis, formed by the union of the lamina transversalis anterior with the nasal septum.
10. The fenestra narinae open directly forward since there is no cupola anterior.
11. The palatine cartilages are directed backwards from the lamina transversalis anterior.

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Explanation of Abbreviations

AH	Ala hypochiasmatica
ATT	Atrioturbinal
BF	Basicranial fenestra
CDN	Cartilago ductus nasopalatini
CRG	Crista galli
CS	Crista semicircularis
DLNG	Duct of lateral nasal gland
FE	Foramen epiphaniale
FO	Optic foramen
HC	Hypophyseal cartilage
IOS	Interorbital septum
JO	Jacobson's organ
LON	Lamina orbito-nasalis
LTA	Lamina transversalis anterior
LTP	Lamina transversalis posterior
MAX	Maxillary bone
MR	Metoptic root of orbital cartilage
MT	Maxillo-turbinal
MXP	Palatine process of maxilla
NAS	Nasal bone
NLD	Nasolachrymal duct
NS	Nasal septum
NT	Nasoturbinal
PAS	Processus alaris superior
PMX	Premaxillary bone
PN	Paranasal cartilage
PR	Preoptic root of orbital cartilage
PS	Paraseptal cartilage
PT	Parietotectal cartilage
SSS	Sulcus Supraseptalis
TG	Tongue
TN	Tectum nasi
VB	Vomer bone

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A Histological Evidence of the Homology between Dahlgren's Cells in Rays and Teleosts

by

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SPEIDEL (1919) proved the glandular properties of Dahlgren's cells in the posterior part of the spinal cord of the rays. According to this author the secretory substances are discharged from the cell bodies into the surrounding tissue and consequently are not transported in the cell processes to a vascularized area as in the diencephalic neurosecretory system or as in the caudal secretory system in the teleosts recently discovered by ENAMI (1955).

The spinal cords of three adult specimens of Trygon violacea were fixed in Bouin's fluid, cut transversally and sagittally in series of 7 microns and stained according to Foot-Masson, Heidenhain's azan and Gomori's chrome alum haematoxylin (1941). Complementary studies were made in Raia radiata, Torpedo ocellata and Leuciscus rutilus treated in the same way as the Trygon material.

In Trygon violacea Dahlgren's cells occupy the spinal cord corresponding to the last 26 vertebrae, forming two rows of cells lateral to the central canal. The largest cells are approximately 140 microns long, 125 microns wide and 65 microns high. The cells have larger processes in the lateral and ventral directions which pass close to the central canal and have extended their course to the ventromedian part of the spinal cord. Here the fissura ventralis is strikingly large and accommodates a richly vascularized part of the endomeninx dorsal to the arteria spinalis. The processes of Dahlgren's cells penetrate the glial membrane of the spinal cord into this vascularized part of the endomeninx. Furthermore the processes branch off to form bulb-like swellings in intimate connection with the vessels and have a more "acidophil" reaction (Figs. 1 and 2). Various part of several cell bodies have an "acidophil" reaction taking azan, fuchsin and phloxine, and processes can be traced by having the same property. In Raia radiata and Leuciscus rutilus the secretion appears as droplets and granules in vacuoles or as plasmatic inclusions in the cells according to SPEIDEL's description. Torpedo ocellata shows considerable variations in the sizes of the cytoplasmatic secretory matter from granules almost at a submicroscopical level to larger droplets. In no case have the secretory products been definitely found free in the surrounding tissue but always bound to the processes forming the classical neurosecretory "perlschnurfasern", and corresponding structures are seen in the processes from the caudal secretory cells in the roach.

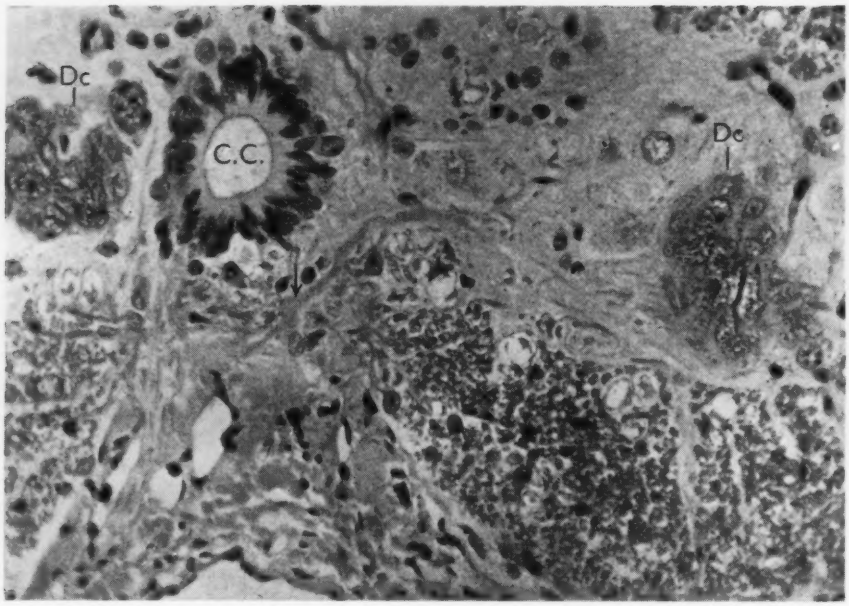

Fig. 1. Ventral to the central canal (c.c.) a proces of a Dahlgren's cell penetrating into the vascularized endomeninx in fissura ventralis (see arrow). To the right and to the left of the central canal Dahlgren's cells (Dc) with their typical extremely polymorphous nuclei. (Trygon violacea, transv., Foot-Masson, 7 microns, 380 ×.)

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Considering the actual state of knowledge concerning the neurosecretory phenomena the observations here reported may solve some problems in SPEIDEL's brilliant studies.

1. SPEIDEL definitively settled that Dahlgren's cells are not electric motor nerve cells and further stated: "If they were nerve cells then we should expect to find the structures which they innervate in this region of the tail. But no special structures of any kind are present here and here only". The histological structure in Trygon violacea clearly shows that the larger processes of Dahlgren's cells "innervate" the vascularized zone of the endomeninx in the fissura ventralis.

2. SPEIDEL observed that most granules pass near the central canal toward the ventral side of the spinal cord and noted here "a network of blood-vessels" and remarked that "Here most of the granules are probably absorbed". He suggested that gravity or the movements of neurolymph may cause this way of secretion. As seen in Trygon violacea the secretory matter is distributed as a homogenous substance in the processes which made it possible to trace them in the course described by SPEIDEL. The transport mechanism is of the same nature as in the neurosecretory systems, a proximodistal flow of plasm.

3. SPEIDEL (1922) could not find sufficient facts to prove the glandular pro-

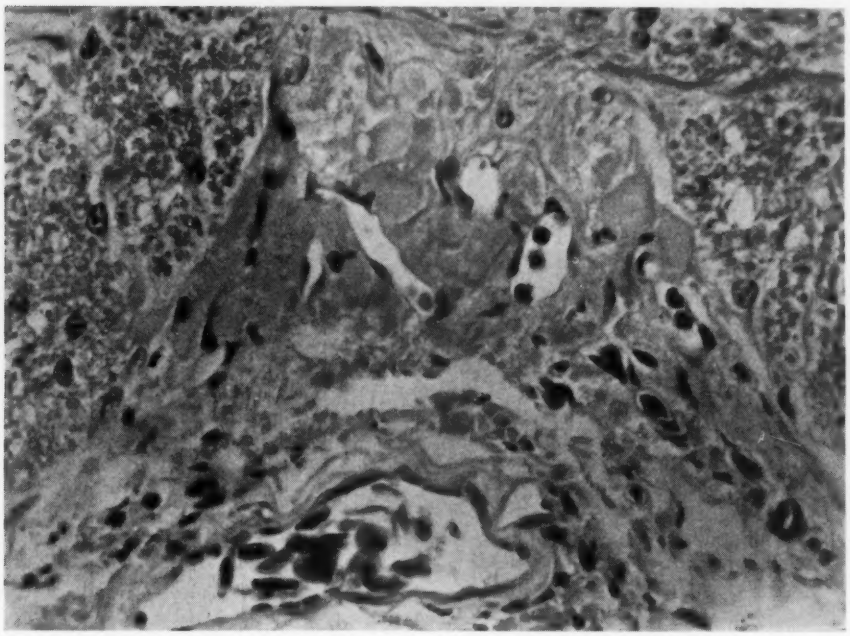

Fig. 2. Terminations of the processes of Dahlgren's cells forming bulb-like "acidophil" swellings in the richly vascularized endomeninx in fissura ventralis. (Trygon violacea, transv., Foot-Masson, 7 microns, 550 ×.)

perties of the large cells in the caudal part of the spinal cord in the teleosts, but, he postulated on the basis of cytological studies, the homology between the cells in rays and teleosts. ENAMI's discovery of the caudal secretory system seemed to establish this homology. However SANO (1954) had observed nerve cells in the ventral gray column of several birds which he supposed to be secretory and which like Dahlgren's cells in rays were said to expel their secretion into the surroundings of the cell bodies. On account of this ENAMI compared these cells in the birds with Dahlgren's cells in the rays as contrasts to the caudal secretory cells in teleosts which like the diencephalic neurosecretory cells transport the secretion in the cell processes. Such a fundamental distinction between different groups within the "Pisces" is not motivated as it has now been demonstrated that the processes of Dahlgren's cells in Trygon violacea function as secretory pathways to a vascularized area in the endomeninx of the fissura ventralis serving the same secretory storing and releasing functions as the terminal enlargement of the spinal cord (Neurophysis spinalis, SANO 1958) in teleosts.

The results obtained confirm two of SPEIDEL's fundamental conclusions. The caudal secretory cells, Dahlgren's cells, are homologous in rays and teleosts and are "gland-cells of internal secretion".

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Das Zentralnervensystem des Castor canadensis, Kuhl.

von

G. PILLERI

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Einleitung

Die morphologische Forschung des Zentralnervensystems des amerikanischen und europäischen Bibers ist äusserst dürftig. Obwohl schon im vergangenen Jahrhundert umfängliche Werke über die Biologie des Bibers verfasst wurden und die gesamte Biberliteratur heute über 1000 Arbeiten umfasst, fehlte eine genauere Untersuchung des Zentralnervensystems bei beiden heute noch lebenden Biberarten stets. Das betrifft aber ebenfalls, wenn auch in geringerem Masse, die Anatomie der übrigen Organe, wobei man sich meistens mit makroskopischen Beschreibungen zufrieden gibt, ohne genaue histologische Untersuchung durchzuführen. Erst in den letzten Jahren, und wie FREYE sagt für den europäischen Biber in letzter Stunde, bemüht man sich (Zoologisches Institut in Halle) den Biber

* Aus dem Hirnanatomischen Institut Waldau/Bern — Schweiz (Prof. E. GRÜNTHAL), dem Department of the Interior Fish and Wildlife Service, Region 4: Atlanta, Ga, USA (Dr. W. A. GRESH, Dr. L. S. GIVENS) und dem Noxubee—National Wildlife Refuge, Miss., USA (B. S. WEBSTER).

anatomisch zu untersuchen, um Beziehungen zwischen Struktur und Funktion, vor allem im Hinblick auf das Wasserleben aufzudecken.

Wir haben uns die Aufgabe gestellt, das Zentralnervensystem des kanadischen Bibers zu untersuchen. Nach einer makroskopischen Beschreibung des Gehirns, so wie man es mit blossem Auge oder unter Zuhilfenahme einer schwachen Leselupe betrachten kann, sollen Arbeiten über die Morphologie des Rückenmarkes, die Neurokrinie im Hypothalamus-Hypophysensystem, die Embryogenese des Cortex und die feinere Anatomie des Zentralorganes (auch an Hand von *Golgi*-Präparaten) folgen. Vergleichend-anatomische Untersuchungen mit den Gehirnen höherer Nagetiere werden den Zweck haben eine mögliche Sonderstellung der zentralnervösen Strukturen bei den *Castoridae* aufzudecken. Ob die Befunde der histologischen und vergleichend-neurologischen Forschung Anhaltspunkte zum Verständnis der besonderen Verhaltenskomplexe des Bibers ergeben werden, kann nur durch die Gesamtbetrachtung der zentralnervösen Strukturen entschieden werden.

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Es sei hier allen, die uns in der Anschaffung der kostbaren Präparate geholfen haben, unsere Dankbarkeit ausgesprochen: Herrn Burton S. Webster, dem Leiter des National Wildlife Refuge in Noxubee (Mississippi, USA), Dr. W. A. Gresh und Dr. L. S. Givens aus dem Dep. of the Interior Fish- and Wildlife Service (Atlanta, Georgia), den Leitern des Dep. of Biology (Miss. State College) Dr. L. L. Ellis, Dr. E. W. Stafford und Dr. D. E. Ferguson. Besonderen Dank gebührt Herrn Professor A. Raspet, dem Leiter des Areophysic Dep., Miss. State College, und seinem Assistenten Dr. Joe Jenkins Cornish III, die uns Flugaufnahmen und Filmen der Bibersiedlungen und Dämme in Noxubee ermöglichten, mit Hilfe eines Piperflugzeuges. Prof. E. Grünthal danke ich für die anregende Teilnahme an dem Projekt und wissenschaftliche Unterstützung; Frl. Margrit Kappeler, Laborantin des Hirnanatomischen Institutes Waldau-Bern, für die technische Hilfe in der photographischen Verarbeitung der Präparate. Prof. Spatz (Max Planck Institut für Hirnforschung — Giessen) ermöglichte uns die Herstellung der Endocranialausgüsse und stellte uns vergleichend-anatomisches Material aus der Sammlung *Brodmann* zur Verfügung. Das Edinger Institut (Prof. W. Krücke) und das anatomische Institut der Universität Frankfurt a/M. (Prof. D. Starck) stellten uns freundlicherweise ihre vergleichend-anatomischen Hirnsammlungen zur Verfügung.

Literatur

Die erste Beschreibung eines Bibergehirnes finden wir in dem Werk von François Leuret und Pierre Gratiolet: "Anatomie comparée du Système nerveux considéré dans ses rapports avec l'intelligence" (Paris 1839—1857). Im Atlas fin-

den wir zwei Zeichnungen eines *Castor*-Gehirnes ohne Artangabe, das folgendermassen beschrieben wird:

"Le cerveau est lisse; au lieu de circonvolutions, il existe seulement sur chaque lobe, une dépression dirigée d'avant en arrière, et une dépression latérale qui est comme un rudiment de la scissure de Sylvius: la première est parallèle à la longueur du cerveau, la seconde est perpendiculaire. Les parties latérales du cervelet, celles qui correspondent au n. 2 du paca et du lapin, sont comparativement plus larges, chez le castor, que chez tous les autres rongeurs, et même que chez la plupart des a autres animaux; la partie moyenne ou ver supérieur, n. 1, est, au contraire, un peu déprimée; quant au lobule n. 3, comme il n'en restait pas de trace sur deux cerveaux de castor, les seuls que j'ai pu examiner, ils manquent dans les figures 1 et 2."

1892 verfasste Frank G. Beddard (Proc. Zool. Soc. London) eine zweite Beschreibung ohne Bilder des *Castor canadensis,* wobei er einige Masse der Hirnlänge und -breite angibt, sich in den Grundzügen aber an das zitierte Werk der Franzosen anschliesst. Eine dritte kurze Beschreibung des Gehirnes von *Castor fiber* verfasste Elliot Smith im Jahre 1902, wobei er aber kaum mehr als die Furchen beschreibt und auf die Arbeit von Beddard verweist. Eine einzige Arbeit aus den dreissiger Jahren von O. Langworthy und C. P. Richter ist neurophysiologisch orientiert und befasst sich mit der Enthirnungsstarre und dem motorischen Cortex bei *Castor canadensis.* Sie enthält keine histologischen Untersuchungen. Das ist alles, was wir bisher über die Anatomie und Physiologie des Bibergehirnes aus der Literatur kennen.

Phylogenie und Systematik, Material

Der Biber gehört zur Ordnung der *Rodentia,* welche mit den Lagomorpha die Superordnung der *Glires* bilden. Phylogenetisch leitet man die Ordnung der Nagetiere von der Ordnung der Insectivora (Tillodontiden — nach E. D. Cope und Schlosser, zit. von M. Weber) ab. Wir haben heute 41 Familien von Nagern, die eine Unzahl Arten umfassen. Nur 4 Familien wären nach den Angaben von J. Viret (Grassé: Traité de Zoologie) ausgestorben: *Mylagaulidae, Ischyromyidae, Eomyidae* und *Cephalomyidae.* Die ungeheure Zahl von Arten und die universelle geographische Verbreitung der Nagetiere beweisen, dass sie heutzutage ihre Blütezeit erleben (M. Weber). Die Familie der *Castoridae* umfasst die Gattungen:

Stenofiber, Geoffroy St. Hilaire
Palaeomys, Kaup
Trogontherium, Fischer
Conodontes, Langel

welche alle ausgestorben sind, und die Gattung *Castor* LIN., die nur mit ihren beiden Arten *canadensis*, KUHL und *fiber*, LIN. erhalten geblieben ist.

Gegenstand unserer Forschung wird der amerikanische Biber, *Castor canadensis*, KUHL sein, der in Nordamerika zahlreiche Rassen bildet. Im Gebiet von Mississippi (*Noxubee*) lebt die Rasse *carolinensis*, RHOADS. In den sumpfigen Gebieten, Morasten und unzähligen Bächen findet der Biber hier als Wassersäuger ideale Lebensbedingungen. Das milde Klima des Landes Mississippi ermöglicht ihm eine viel längere Arbeitszeit im Jahre als seinen Artgenossen in den kälteren kanadischen Regionen. Gewaltig sind die Werke des Bibers im *Bluff*-Lake, der zahlreiche Dämme, Biberburgen mitten im Wasser oder Banklodges enthält, sowie komplizierte Schwimmwege in Form von Kanälen an seinem Ufer aufweist. Die Gesamtbevölkerung des *Noxubee*-Refuge wird auf 2000 Biber geschätzt (B. S. WEBSTER).

Für die vorliegende Untersuchung haben wir 12 Biber dieser Rasse aus verschiedenen Altersstufen verwendet. Die folgende Tabelle gibt einen Überblick über die Körpermasse und Gewichte und weist auf die starke individuelle Variabilität dieser Masse hin.

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Tabelle 1

SNr.	Geschlecht	Körpergewicht cm	Körperlänge cm	Schwanzlänge cm	Schwanzbreite cm	Schwimmfüsse Länge cm	Bemerkungen
T 21	♂	20,5	70	32	—	17	
T 22	♂	18	68	30	—	17	
T 23	♂	14,5	55	30	13	15	
T 24	♂	22,5	64	32	14	16	
T 25	♀	27	—	34	17	18	trächtig
T 26	♂	8	47	16	6	10	
T 27	♂	—	50	25	12	—	
T 28	♂	9,75	54	28	12	11	
T 29	♂	12,5	65	33	15	16	
T 30	♂	16	72	30	14	18	
T 31	♂	22,5	70	30	14	17	
T 32	♀	5,5	48	25	—	14	

Die Tiere sind durch Gewehrschuss ins Abdomen, meist schwimmend bei Sonnenuntergang, erlegt und die Gehirne kurze Zeit nach dem Tode präpariert worden. Nach Reinigung der Schädelkalotte bis zu den Jochbögen wird zwischen beiden Augen das Frontalbein durchgesägt. Ein Sägeschnitt wird möglichst weit lateral an der Ansatzstelle des Processus zygomaticus beidseits durchgeführt, mit dem frontalen Querschnitt nach vorne verbunden und nach kaudal gegen den knöchernen Meatus acusticus (1 cm medial davon) hin verlängert, durch die querverlaufenden Cristae des Hinterhauptbeines und von hier nach oral gegen

das Foramen occipitale magnum geführt. Mit Hilfe einer flachen Knochenzange wird die Schädelkalotte am besten in mehreren kleinen Portionen vorsichtig von der Dura abgehoben, welche bei älteren Tieren straff mit des Schädelkalotte verwachsen ist. Die Dura wird rechts entlang des Sinus sagittalis superior und median über das Kleinhirn gespalten und samt Falx und Tentorium vom Gross- und Kleinhirn und der Oblongata enfernt. Das Rückenmark wird in der Höhe von C_1—C_2 durchgeschnitten, nach Laminektomie der entsprechenden Wirbelbögen. Mittels einer Hirnspatel wird das Spatium subdurale in allen zugänglichen Gebieten unterminiert und das ganze Gehirn samt den Bulbi olfactorii lateral umfahren. Die Knochennische des Flocculus wird mit der Zange gesprengt. Mit Pinzette und Schere wird das Rückenmark und die Oblongata an den Nervenwurzeln möglichst distal präpariert und von der Unterlage des Clivus abgehoben. Mit einem Messer wird die Dura in der Höhe des vorderen Brückenrandes (kaudale Naht des Os basisphenoideum) inzidiert und die Hypophyse unterminiert, welche völlig flach in einer extrem seichten Sella der Schädelbasis aufliegt. Von hier wird entlang des Trigeminus mit einer gebogenen Schere nach vorne und medial präpariert. Die Nervi optici, sowie alle Hirnnerven werden distal abgeschnitten.

Die meisten für diese makroskopische Studie verwendeten Gehirne sind in neutraler Formollösung 1 : 9 konserviert worden. Bei den Körpersektionen konnten mit blossem Auge an den Organen keinerlei pathologische Veränderungen konstatiert werden. Einige alte Männchen wiesen vernarbte Kellendefekte auf. Nur T-Nr. 22 wies einige eingekapselte Abszesse in der Subcutis auf.

Körper- und Hirngewicht

Die nachfolgende Tabelle gibt unsere Masse bei 8 *Castor canadensis* wieder. Das Hirngewicht wurde am fixierten Präparat festgestellt, das Körpergewicht am frischen Tier genommen.

Tabelle 2

SNr.	Geschlecht	Körpergewicht gr	Hirngewicht gr	Hirngewicht (=1) zu Körpergewicht
T 24	♂	22500	52	1 : 433
T 21	♂	20500	44	1 : 466
T 22	♂	18000	53	1 : 320
T 30	♂	16000	38	1 : 421
T 23	♂	14500	45	1 : 322
T 29	♂	12500	42	1 : 293
T 28	♂	9750	48	1 : 203
T 26	♂	8000	38	1 : 211

Wenn man die Durchschnittswerte ausrechnet, verhält sich das Hirngewicht (= 1) zum Körpergewicht ca. wie

1 : 330.

Allgemeine Formverhältnisse des Gehirnes

Dorsal betrachtet (Abb. 1, A) bilden die Gross- und Kleinhirnkonturen annähernd eine ovale Form, die rostral gegen die Frontalpole* in einem Winkel von etwa 60° zugespitzt erscheint. Die maximale Breite der Grosshirnhemisphären findet sich in der Mitte der Hemisphärenlänge. Auch in kaudaler Richtung spitzen sich die Hemisphären des Grosshirns etwas zu unter Bildung eines deutlichen Occipitalpols, der vor den Paraflocculi zu liegen kommt. Die kaudalen Hemisphärenkanten bilden miteinander einen stumpfen Winkel von etwa 150° und überdecken leicht die antero-dorsalen Partien des Kleinhirns. Die Übergangsstelle der kaudalen zur sagittalen Mantelkante ist abgerundet, und man kann in der Tiefe das Tuberculum posterius der Vierhügelplatte erblicken. Diese kaudo-sagittale Rundung ist variabel und bei manchen Individuen erscheint die Vierhügelplatte vollkommen überdeckt und dorsal nicht sichtbar. Der Frontalpol ist abgerundet und deckt einen kurzen kaudo-dorsalen Teil des Bulbus olfactorius. Paramedian, der sagittalen Mantelkante fast parallel verlaufend, findet sich in jeder Hemisphäre die schon von LEURET und GRATIOLET (op. cit.) beobachtete Längsfurche. Sie weist bei manchen Gehirnen einen leicht zick-zack artigen Verlauf oder kleine Unterbrechungen auf. Manchmal haben die Furchen die Tendenz in ihrer vorderen Hälfte zu divergieren. Ihre Länge ist auch Schwankungen unterworfen; wir messen von 15 bis 22 mm, unabhängig von der Grösse des Gehirnes, dem Geschlecht oder Alter des Tieres. Diese Furche ist nicht etwa durch den Verlauf eines Gefässastes bedingt, ihr Grund ist meistens gefässfrei oder enthält nur winzige Gefässe. Ihrer Lage entsprechend finden wir auf der endocranialen Fläche der Parietalknochen zwei gleich grosse Juga cerebralia (siehe cranio-cerebrale Beziehungen). Die gesamte dorsal sichtbare Rinde des Grosshirnes gehört dem *Neocortex* an. Das Kleinhirn hat dorsal betrachtet einen gut ausgebildeten Wurm und breite Hemisphären. Die Paraflocculi überragen seitlich die Breite der Grosshirnhemispräre nicht. Die Medulla oblongata wird vom Kleinhirn nicht vollständig überdeckt, wobei hier wieder die gegenseitigen Beziehungen leichten Schwankungen unterstehen. Die Cysterna cerebello-medullaris ist geräumig.

Bei *seitlicher* Betrachtung (Abb. 2, A) ist die Rundung des Frontalpoles und dessen Beziehung zum Bulbus olfactorius deutlich. Das Grosshirn ist dorso-oral eher abgeplattet. Die laterale Fläche der Grosshirnhemisphären ist glatt, im Ver-

* Die Bezeichnungen frontaler und occipitaler Pol sind hier nur *makroskopisch-topographisch* verwendet worden, entsprechend der Lage gleichbenannter Schädelknochen. Das Wort Frontalpol setzt also nicht voraus, dass hier auch eine Frontalrinde im *cytoarchitektonischen* Sinne vorkommt.

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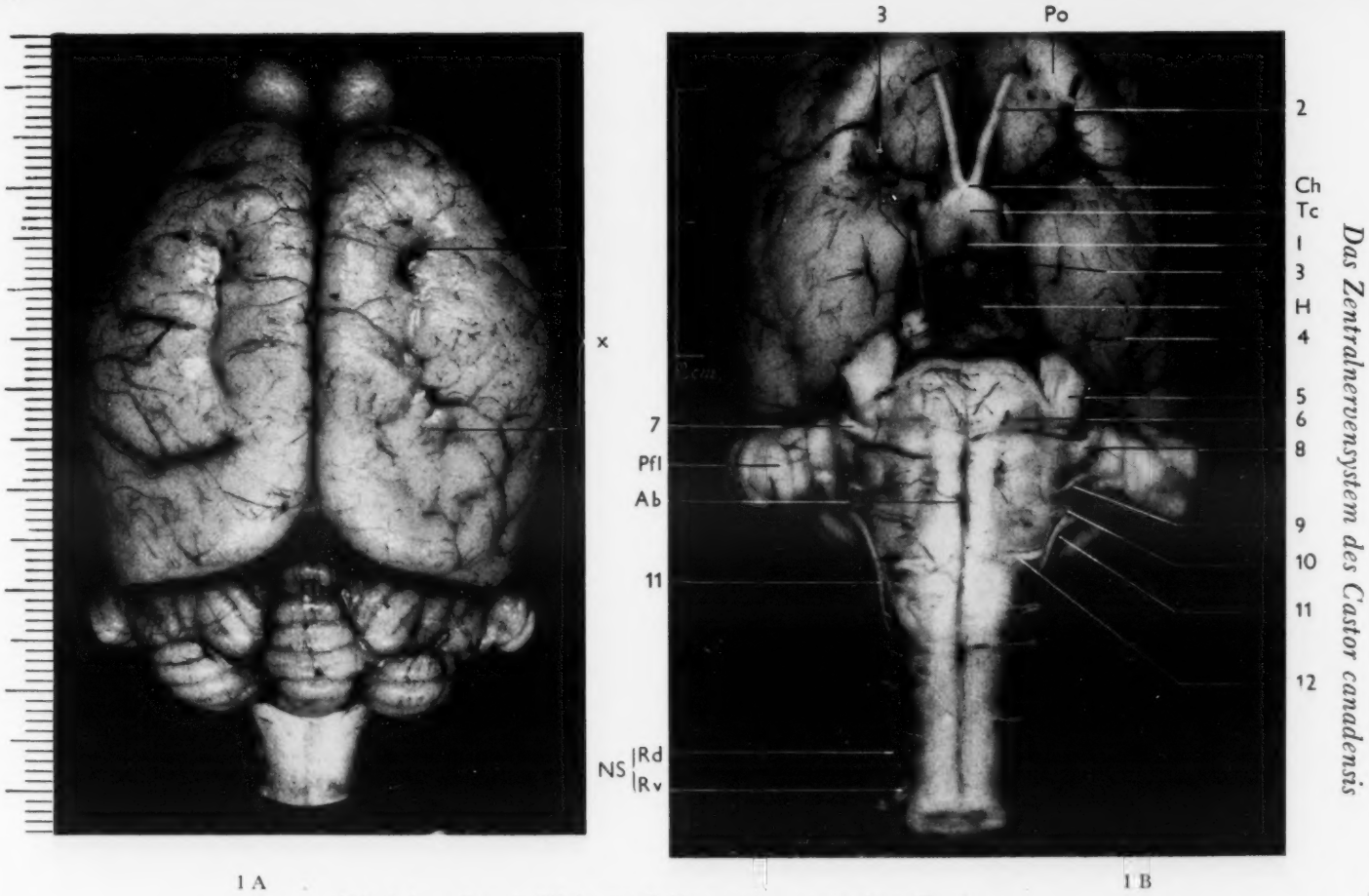

Abb. 1 A — *Dorsale Fläche des Gehirnes:* × = parasagittale Furche.

Abb. 1 B — *Basale Fläche des Gehirnes:* Po = Pedunculus olfactorius, 2 = Nervus opticus, Ch = Chiasma, Tc = Tuber cinerum, I = Infundibulum, 3 = Nervus oculomotorius, H = Hypophyse (Adenohypophyse), 4 = Nervus trochearis, 5 = Nervus trigeminus, 6 = Nervus abducens, 7 = Nervus facialis, 8 = Nervus stato-acusticus, 9 = Nervus glossopharyngicus, 10 = Nervus vagus, 11 = Nervus accessorius, 12 = Nervus hypoglossus, Pfl = Paraflocculus, Ab = Arteria basilanis, Ns = Nervus spinalis mit Radix dorsalis (Rd) und Radix ventralis (Rv), → = Fissura intrapyriformis.

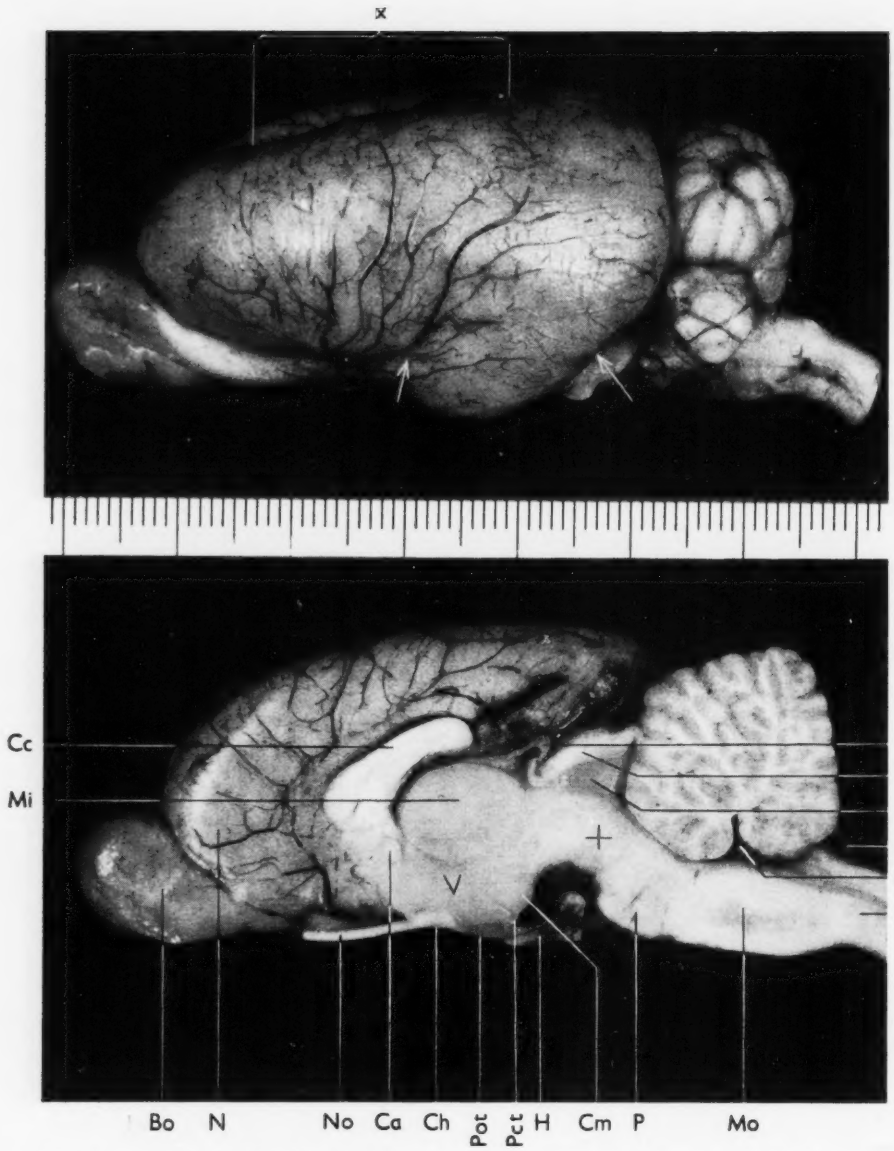

Abb. 2 A — *Laterale Fläche des Gehirnes:* × = parasagittale Furche, die Pfeile zeigen den Verlauf der Fissura rhinalis (palaeo-neocortikale Grenze).

Abb. 2 B — *Mediansagittalschnitt durch das Gehirn:* Cc = Corpus callosum, Mi = Massa intermedia, Bo = Bulbus olfactorius, N = Neocortex, No = Nervus opticus, Ca = Commissura anterior, Ch = Chiasma, Pot = Pars oralis tuberis, Pct = Pars caudalis tuberis, H = Hypophyse, Cm = Corpus mammillare, + = Mittelhirn, V = III. Ventrikel, E = Epiphyse. Lq = Lamina quadrigemina, A = Aquaeductus Sylvii, Cem = Cisterna cerebello—medullaris, P = Pons, R = Rautengrube, Mo = Medulla oblongata, Ms = Medulla spinalis.

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teilungsgebiet der Arteria cerebri media sehr leicht eingedellt, jedoch *ohne* Bildung einer Vallecula Sylvii. Der Occipitalpol liegt in der Höhe des Paraflocculus, welcher ser gut ausgebildet ist. Die Verhältnisse im Kleinhirn und in der Oblongata sind aus dem Bild ersichtlich. Von den basalen Formationen sind der Bulbus und Pedunculus olfactorius gut zu sehen. Die Fissura rhinalis (= Fissura palaeoneocorticalis nach SPATZ) beginnt als Fissura rhinalis posterior kaudal zwischen dem Occipitalpol und der oralen Wölbung des Lobus pyriformis und ist nur bei lateraler oder basaler Betrachtung des Gehirnes sichtbar. Die Fissura rhinalis posterior trennt den Neocortex vom Lobus pyriformis (Palaeocortex) ab und setzt sich, seitlich von der Stria olfactoria lateralis und dem Pedunculus olfactorius verlaufend, als Fissura rhinalis anterior in kranialer Richtung fort. Der schmale Gyrus olfactorius lateralis ist infolge der Ausdehnung der Frontalgebiete von der Seite nicht sichtbar. Das Tuberculum olfactorium springt oral wenig vor und ist von der Seite kaum zu sehen.

Bei *basaler* Betrachtung (Abb. 1, B) verläuft die Fissura rhinalis anterior sehr medial, wodurch grosse Teile des frontalen Neocortex eine ventrale Lage einnehmen. Die Bulbi entsenden einen starken Pedunculus olfactorius, dem sich seitlich ein schmaler Gyrus olfactorius lateralis anschliesst. Der Gyrus olfactorius medialis geht in ein breites und abgeflachtes Tuberculum olfactorium über. Der Sulcus endorhinalis (=Sulcus arcuatus), die anterolaterale Abgrenzung des Tuberculum, ist vorne gegen den Gyrus olfactorius medialis sehr seicht, an der Grenze der Stria olfactoria medialis hingegen tief. Caudal vom Tuberculum olfactorium finden wir ein schmales Spatium parolfactorium basale, das ohne Sulcus rhinicus transversus (eine bei den Carnivoren beschriebene Furche) in den Lobus pyriformis übergeht. Dieser ist medial gegen das Tubergebiet des Zwischenhirns in Form einer „Uncus"-artigen Bildung — wir möchten die Bezeichnung „Uncus pyriformis" vorschlagen — ausgebuchtet, welche von einer kurzen, *konstant* vorkommenden, etwa 4—5 mm langen, tiefen Furche lateral abgegrenzt erscheint (intrapyriforme Furche). Über die Bedeutung dieser mediobasalen intrapyriformen Furche wird die vergleichend-anatomische Untersuchung entscheiden. Die Fissura rhinalis posterior wird in der Höhe der maximalen Entfaltung des Lobus pyriformis undeutlich. Kaudaler wird sie immer tiefer und schnürt direkt den hintern Pol des Neopalliums und den restlichen Neocortex von der kaudalen Fläche des Lobus pyriformis (Palaeocortex) ab (Abb. 4).

Die Abbildung 2 B stellt die *Medianfläche* des Gehirns dar. Rostral liegt der Bulbus olfactorius, der sich scharf mit einem Sulcus vom Grosshirn abgrenzt. Der Balken liegt tief, weit von der Mantelkante entfernt und ist kurz und dick gestaltet. Oral vom Balkenknie finden sich eine gut ausgebildete Commissura anterior, die Columna fornicis, die lateral-vorne das Foramen Monroi abgrenzt, und eine sehr breite Massa intermedia thalami. Zwischen dieser und dem Zwischenhirnboden liegt der III. Ventrikel. Dieser ist vorne durch die Lamina terminalis abgeschlossen. Oral bildet sich ein seichter Recessus supratopticus und infundibuli.

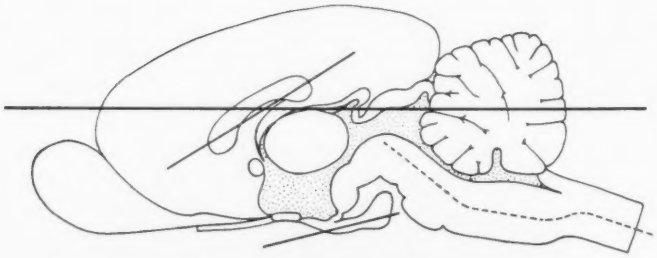

Abb. 3. Gegenseitige Beziehungen zwischen den Hirnachsen.

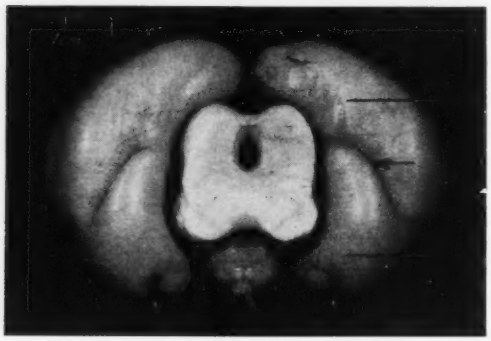

Abb. 4. *Kaudale Grosshirnfläche:* N = Neocortex, P = Palaeocortex, ← = Fissura rhinalis, ↑ = Fissura intrapyriformis.

Am Hypothalamus selber kann man Tuber, Hyophyse und ein markreiches Corpus mammillare erkennen. Die Längsachsen der beschriebenen Formationen und ihre gegenseitige Lage sind im Schema Abb. 3 illustriert. Ein Vergleich mit der Situation bei anderen Nagetieren und den übrigen Säugern erfolgt im vergleichend-anatomischen Beitrag. Dorso-kaudal der Massa intermedia liegt die Epiphyse, mit einem weiten Recessus epiphyseos. Oral davon findet sich die graue Habenula. Die Commissura posterior ist schmal. Der Epiphysenachse parallel ist auch die Lamina quadrigemina orientiert, welche schräg dachförmig einen sehr weiten Aquaeductus Sylvii überdeckt. Die Mittelhirnachse ist entgegengesetzt orientiert und beschreibt einen nach oben flach konkaven Bogen um den Vermis cerebelli, der die Fossa rhomboidea überdeckt. Die Brücke ist kurz im Verhältnis zur Medulla oblongata, die flach und lang ist.

Rolf Schneider hat in seinen „Morphologischen Untersuchungen über das Gehirn der Chiroptera" (1957) Winkelmessungen zum Erfassen der Formänderungen der Grosshirnhemisphären bei verschiedenen Arten eingeführt. Sie sind den Indexwerten vorzuziehen, da „ein Winkel — zwar auch nur Ausdruck des Verhältnisses zweier Strecken zueinander — ohne weiteres dieses Verhältnis erkennen lässt und m. E. wesentlich anschaulicher als eine den Index repräsentierende Zahl

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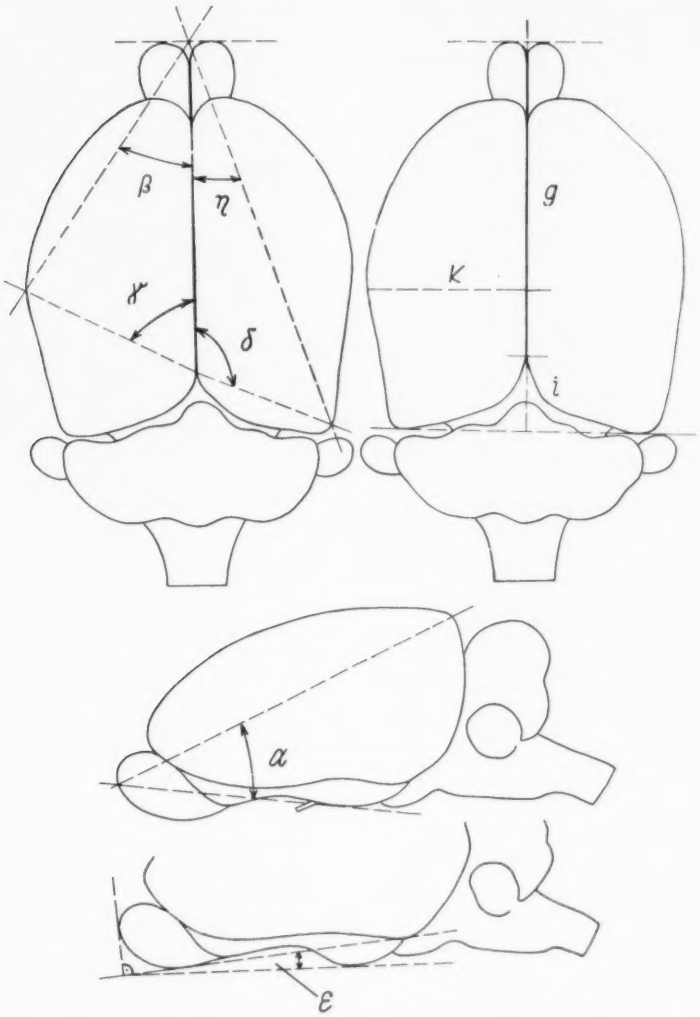

Abb. 5. Schematische Darstellung der Winkel- und Indexmessungen nach *R. Schneider.*

ist" (R. SCHNEIDER). Wir wollen in Anbetracht der späteren vergleichend-anatomischen Forschung die Masse dieser Winkel für das Bibergehirn angeben. SCHNEIDER misst einen Winkel α als Mass der Stauchung des Palliums, einen Winkel β und γ zur Bestimmung der seitlichen Ausladung, einen Winkel δ und η zur Bestimmung der occipitalen Verschiebung. Mit zwei Indices bestimmt er ausserdem die Streckenverhältnisse der seitlichen Ausladung und der Occipitalverschiebung des Palliums. Die Schemata Abb. 5 machen diese Messmethode ersichtlich. Folgende Tabelle gibt die Masse bei 8 Gehirnen von Castor canadensis wieder.

Tabelle 3

SNr.	α	β	γ	δ	η	ε	$\frac{K \cdot 100}{g}$	$\frac{i \cdot 100}{g}$
T 21	33	36	59	112	20	4	50,5	17,4
T 22	—	35	62	115	20	—	51,1	21,5
T 23	—	35	64	116	20	—	52,8	20,0
T 24	34	32	66	118	19	5	52,3	25,7
T 26	31	—	—	—	—	4	—	—
T 27	34	38	62	114	20	4	56,0	21,1
T 28	32	38	62	118	20	5	56,0	23,8
T 32	—	38	60	120	18	—	52,0	25,3

Hypothalamus-Hypophysensystem

Der Hypothalmus oder besser ausgedrückt das Hypothalamus-Hypophysensystem hat in den letzten Jahren auch morphologisch eine solche Bedeutung erfahren (BARGMANN, SPATZ), die uns veranlasst, vor allem im Hinblick auf die spätere mikromorphologische Untersuchung, es schon makroskopisch genauer zu betrachten. Wir wählen bei der Beschreibung die Nomenklatur, die SPATZ für dieses Hirngebiet eingeführt hat; sie ist, was Klarheit anbelangt, die beste. Basal gesehen (Abb. 6) finden wir unmittelbar kaudal vom Chiasma eine *ausgedehnte* Pars oralis tuberis (3—4 mm lang), an der sich ein sehr kurzes, proximal etwa 3 mm breites Infundibulum anschliesst. Der Ansatz des Hypophysenstiels erfolgt im hinteren Drittel des Hypothalamus, ein Befund, der dem von BECKER für die Muriden entspricht. Lateral vom Hypophysenstiel finden wir eine Pars parainfundibularis. Die Hypophyse (Abb. 7) ist schaufelförmig, und reicht mit ihrem kaudalen Ende bis zum freien Brückenrand. Die Neurohypophyse dehnt sich mit einem aboralen Zipfel in die tiefe Fossa interpeduncularis aus. Die Fossa wird bei basaler Betrachtung fast ganz von der Adenohypophyse überdeckt. Die Neurohypophyse ist nur bei dorsaler Betrachtung des Organs sichtbar und ist in der Adenohypophyse mit dem grössten Teil des Infundibulum eingebettet. Trennt man die Hypophyse ganz proximal am Stiel ab, dann findet man eine *sehr kurze,* mehr aufrecht gestellte Pars caudalis tuberis (Abb. 7 a) und ein breites, dorsal davon liegendes, unpaariges Corpus mammillare, das im Längsschnitt sehr markfaserreich ist.

Die Grösse der Hypophyse ist deutlichen Schwankungen unterworfen, die nur zum Teil von der Grösse des Gehirnes oder dem Körpergewicht abhängig sind. Wir haben den Eindruck, dass das Alter bestimmend auf die Dimensionen der Drüse wirkt, wobei hier die Untersuchung eines umfänglichen Materials notwendig wäre. Wir konnten bei 6 Gehirnen von männlichen Bibern folgende Masse nehmen (Tab. 4):

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Tabelle 4

SNr.	Körpergewicht kg	Hypophyse		
		Länge mm	Breite mm	Höhe mm
T 24	22,5	11	13	—
T 21	20,5	9	9,5	—
T 22	18	11	13	5,5
T 23	14,5	9	10	—
T 28	9,75	9	9,5	—
T 26	8	9	8,7	5

Abb. 6. *Mittleres basales Hirnbereich:* Fra = Fissura rhinalis anterior, Gom = Gyrus olfactorius medialis, Po = Pedunculus olfactorius, To = Tuberculum olfactorium, Sd = Sulcus diagonalis, Se = Sulcus endorhinalis, Spb = Spatium parolfactorium basale, Pot = Pars oralis tuberis, To = Tractus opticus, Pp = Pars parainfundibularis, I = Infundibulum, Cm = Corpus mammillare, Fi = Fissura intrapyriformis, Pc = Pedunculus cerebri, Fip = Fossa interpeduncularis, 3 = Nervus oculomotorius.

Abb. 7. *Hypophyse* (dorsal, Mediansagittalschnitt): I = Infundibulum, N = Neurohypophyse, A = Adenohypophyse, S = laterale adeno-neurohypophysäre Grenze, K = neuro-adenohypophysäre Kontaktfläche.

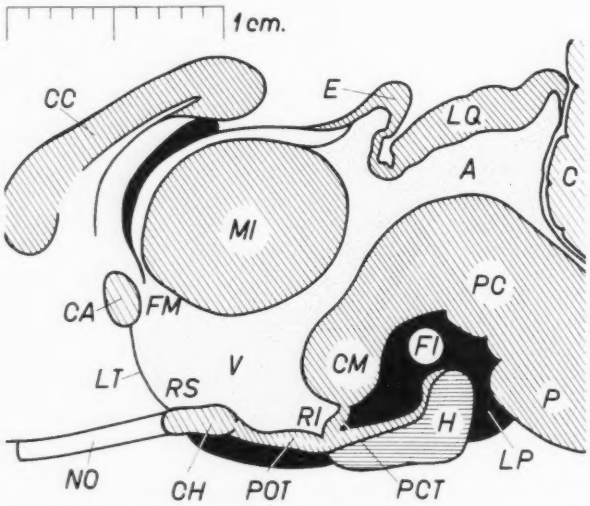

Abb. 7 A. *Mediansagittalschnitt durch das Zwischenhirn* (Schematisch): CC = Corpus callosum, E = Epiphyse, LQ = Lamina quadrigemina, C = Kleinhirn, A = Aquaeductus Sylvii, MI = Massa intermedia, FM = Foramen Monroi, CA = Commissura anterior, LT = Lamina terminalis, V = III. Ventrikel, RS = Recessus supraopticus, NO = Nervus opticus, CH = Chiasma, POT = Pars oralis tuberis, RI = Recessus infundibuli, PCT = Pars caudalis tuberis, CM = Corpus mammillare, H = Hypophyse, FI = Fossa interpeduncularis, PC = Pedunculus cerebri, P = Pons, Lp = Lobus pyriformis (rechter).

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Thalamus

Auf Seite 113 schon beschriebenen Medianfläche zeigt der Thalamus unterhalb der Massa intermedia keine Abgrenzung zum Hypothalamus. Rostral berührt die Massa intermedia den Fornix, dorsal wird der Thalamus zum Teil vom Balken überdeckt, wobei die Epiphyse vom Balken vollkommen unbedeckt bleibt. Bei der stratigraphischen Präparation von dorsal her (Abb. 8) unter Abkappung der Grosshirnhemisphären und des Balkens mit einem Teil der Commissura hippocampi, ist die Form des Thalamus deutlich zu sehen. Er ähnelt einem Dreieck, dessen kaudale Kathete die Grenze zum Mittelhirn darstellt, die mediale Kathete die Habenula und die Hypotenuse die seitliche Rundung darstellt. Der laterokaudale Winkel wird vom stark vorspringenden Corpus geniculatum (wahrscheinlich *laterale,* die genauere Bezeichnung erfolgt histologisch), der medio-kaudale von der Epiphyse und der rostrale Winkel vom Tuberculum anterius gebildet. An die laterale Rundung legt sich schalenartig der Archicortex mit seinen Fasersystemen an. Die Wölbung der dorsalen Fläche des Thalamus ist einheitlich, die Bildung von Tubercula ist nicht ausgeprägt. Zwischen Thalamus und Nucleus caudatus findet sich eine deutliche Lamina affixa und im Sulcus optostriatus (*Beccari*) zeichnet die sehr breite Stria terminalis die ventrikuläre Grenze zwischen Striatum und Thalamus.

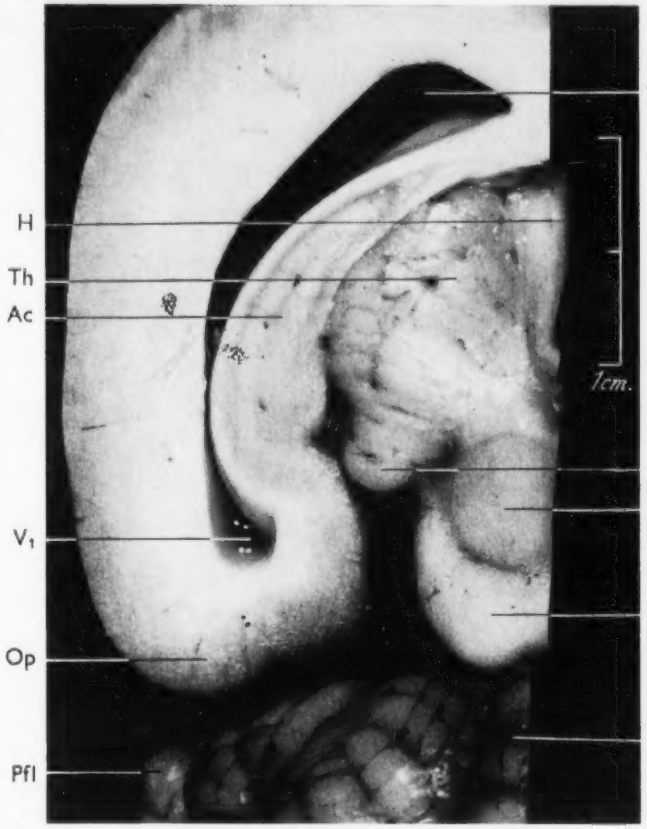
Mittelhirn

Oral betrachtet wird das Mesencephalon fast ganz von der Adenohypophyse bedeckt, die der Fossa interpeduncularis aufliegt (Abb. 1 B). Nach Abtragung der Drüse sieht man die Pedunculi cerebri, den Ursprung des Nervus oculomotorius und die tiefe Fossa interpeduncularis.

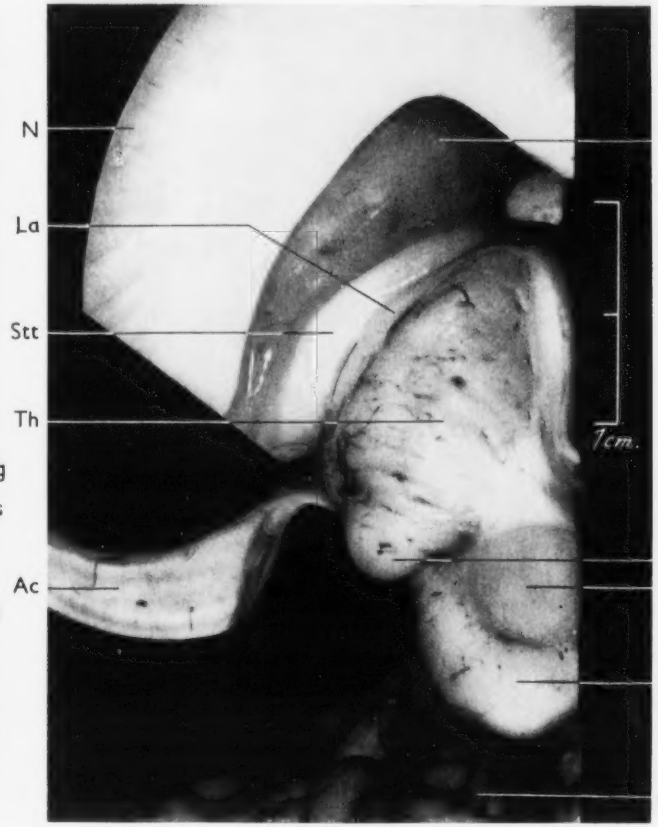
Dorsal sind nur bei manchen Individuen, bei welchen die medio-kaudalen Hemisphärenkanten stärker klaffen, die Colliculi inferiores der Lamina quadrigemina sichtbar.

Bei Lateralansicht ist beim intakten Gehirn vom Mittelhirn nichts zu sehen.

Die Lamina quadrigemina (Abb. 8 A) wird durch Abpräparieren der occipitalen Partien der Grosshirnhemisphären freigelegt. Die Colliculi superiores sind rund, schön gewölbt, an der Oberfläche grau gefärbt und gut von der Umgebung abgegrenzt. Die Grenze zum Thalamus wird durch eine sehr leichte Eindellung und vor allem durch den sehr scharfen Farbunterschied erkannt. Die Colliculi inferiores sind in der Längsausdehnung kürzer, von weisser Farbe und gehen in das Brachium colliculi inferioris über. Bei der lateralen Freilegung des Mittelhirnes kann man höchstens eine leichte Vorwölbung des Brachium colliculi inferioris wahrnehmen, die sich in den Pedunculus cerebri verliert. Sonst findet man wenig Relief an der Aussenfläche; das Trigonum lemnisci wölbt sich etwas vor und ist



8 A



8 B

Abb. 8 A — *Nach Entfernung von dorsalen Grossbirnteilen sind der Thalamus und das Mittelhirn freigelegt:* V = Seitenventrikel, H = Habenula, Th = Thalamus, Ac = Archicortex, V_1 = Ventrikel (Hinterhorn), Cg = Corpus geniculatum, Cs = Colliculus superior, Ci = Colliculus inferior, C = Cerebellum, Op = Occipitalpol, Pfl = Paraflocculus.

Abb. 8 B — *Der Hippocampus ist entfernt worden:* V = Seitenventrikel, N = Neocortex, La = Lamina affixa, Stt = Stria terminalis, Th = Thalamus, Ac = Archicortex, Cg = Corpus geniculatum, Cs = Colliculus superior, Ci = Colliculus inferior, C = Cerebellum.

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vom Pedunculus cerebri durch einen schrägen Sulcus abgegrenzt (Abb. 9). Zwischen dem Velum medullare und den Colliculi inferiores entspringt der Nervus trochlearis, der entlang des Brachium conjunctivum und pontis die Hirnbasis erreicht.

Brücke

Sie ist kurz und quer gelagert. Der freie Rand wird bei manchen Gehirnen mit grosser Hypophyse von deren kaudalem Rand berührt. Die kaudale Grenze der Brücke ist nicht sehr deutlich, entspricht ungefähr der Verbindungslinie zwischen den Facialiswurzeln, bzw. der cranialen Abgrenzung der Pyramidenwölbungen. Auf dem Mediansagittalschnitt ist die Grenze zur Oblongata deutlicher zu sehen (Abb. 2 B). Lateral zwischen Pons und Kleinhirn treten die mächtigen Wurzeln des Nervus trigeminus aus. Die Basismitte des Pons ist längs eingedellt, entsprechend dem Verlauf der Arteria basilaris und der Fortsetzung der Fissura mediana anterior der Medulla oblongata.

Medulla oblongata

Sie ist etwa doppelt so lang wie die Brücke und rostral fast so breit; sie verjüngt sich allmählich in kaudaler Richtung. Gegen das Rückenmark zeigt sie eine leichte, laterale Einschnürung. Ventral ist sie durch die Fissura mediana anterior längs gefurcht. Lateral von dieser liegen die Pyramiden. Die Oliven sind nicht vorgewölbt.

Aus der Medulla entspringen die meisten Hirnnerven. Wir erkennen (Abb. 1 B) lateral vom Olivengebiet in einer Reihe angeordnet den gut ausgebildeten Nervus stato-acusticus, glossopharyngicus, vagus und die kranialen und kaudalen Wurzeln des accessorius. In einer medialen Reihe angeordnet findet sich an der Grenze Brücke-Oblongata der Nervus accessorius und zwischen Olive und Pyramide die Wurzeln des Nervus hypoglossus.

Dorsal bildet die Medulla oblongata die Rautengrube, den Boden des IV. Ventrikels. Die in der Abb. 10 B hervortretenden Formationen benötigen eine mikroskopische Untersuchung. Von einer makroskopischen Benennung wird aus diesem Grund vorläufig Abstand genommen. Die topographische Lage der Corpora restiformia, Brachia pontis und conjunctiva ist aus demselben Bild ersichtlich.

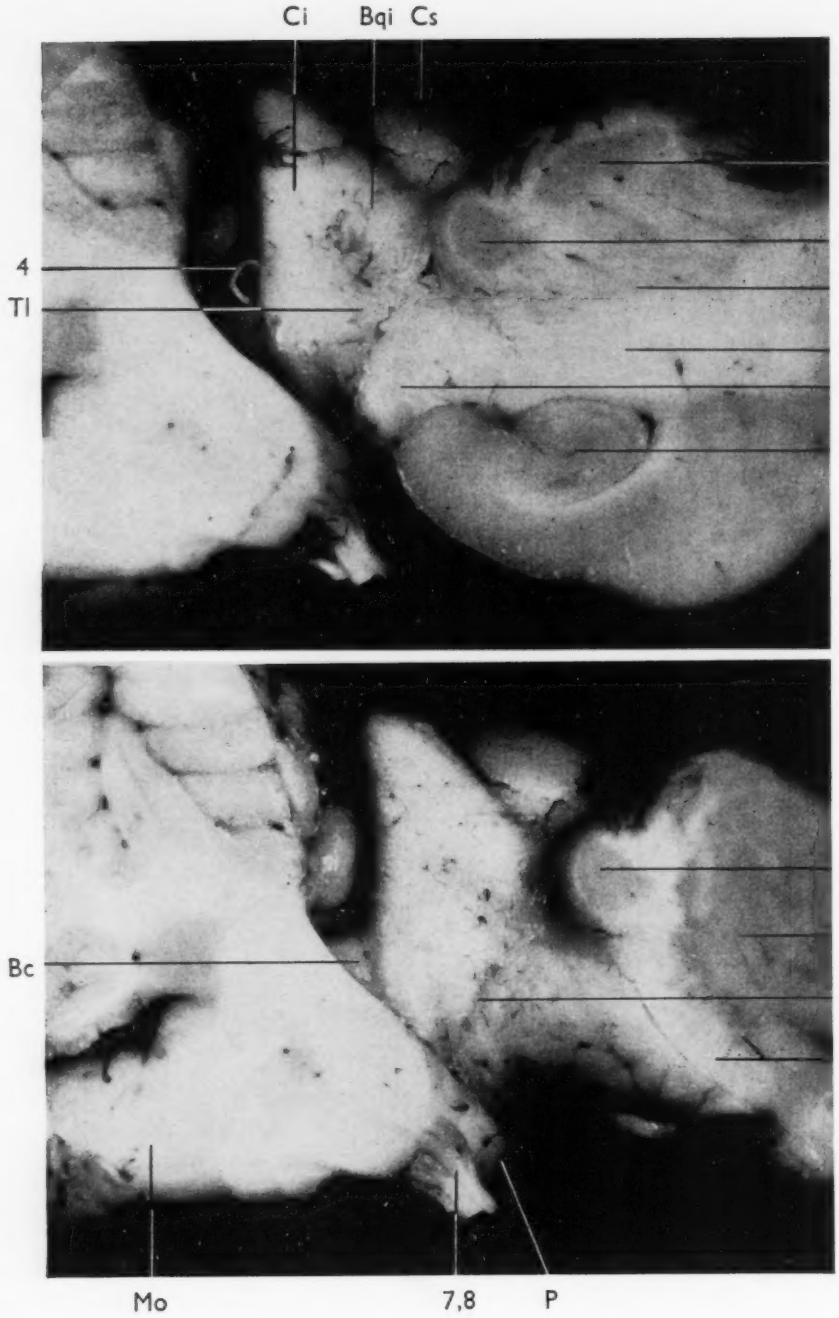

Abb. 9. *Mittel- und Zwischenhirngebiete.* Bild oben: Ci = Colliculus inferior, Bqi = Brachium quadrigeminum inferius, Cs = Colliculus superior, Cg_1 = Corpus geniculatum(?), Cg = Corpus geniculatum, Th = Thalamus (Lateralkerne), Ci = Capsula interna, 4 = Nervus trochlearis, Tl = Trigonum lemnisci. Bild unten: Cg = Corpus geniculatum, Th = Thalamus, S = Sulcus vor dem Trigonum lemnisci, Pc, Sn = Pedunculus cerebri, Substantia nigra (Schräg frontal geschnitten), Bc = Brachium conjunctivum, P = Pons, 7, 8 = Nervus facialis, Nervus stato-acusticus, Mo = Medulla oblongata.

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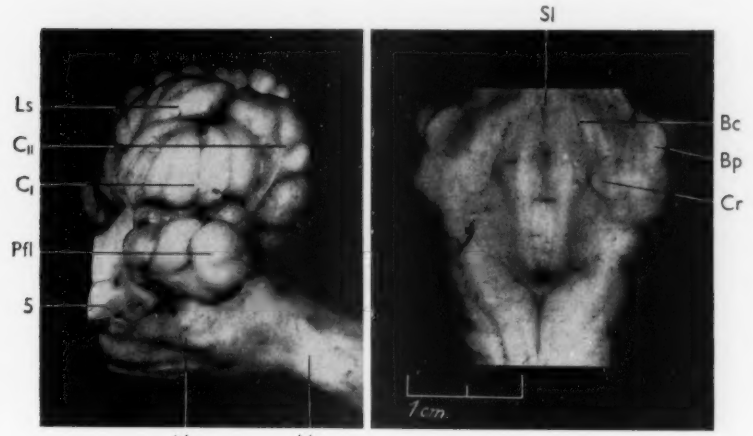

10 A 10 B

Abb. 10 A — Laterale Kleinhirnfläche: Ls = Lobulus simplex, C_{II} = Crus secundum, C_I = Crus primum, Pfl = Paraflocculus, 5 = Nervus trigeminus, Mo = Medulla oblongata, Ms = Rückenmark.

Abb. 10 B — *Rautengrube:* Sl = Sulcus longitudinalis, Bc = Brachium conjunctivum, Bp = Brachium pontis, Cr = Corpus restiforme.

Kleinhirn*

Dorsal (Abb. 11 B) betrachtet unterscheidet man rostral, hinter der Fissura prima, den Lobulus simplex, welcher im Bereich der Hemisphären mächtig geschwollen ist. Seitlich von dieser Anschwellung liegt das Crus I. Kaudal vom Lobulus simplex findet sich der Lobulus medius mit einem sehr gut entwickelten Crus II, an das sich lateral der Paraflocculus anschliesst. Die Uvula ist bei der dorsalen Betrachtung kaum sichtbar.

Rostral (Abb. 11 A) sehen wir median den Lobulus centralis und das Culmen. Lateral erreicht die Fissura prima die Kleinhirnschenkel. Dorso-lateral findet sich die seitliche Anschwellung des Lobulus simplex und noch seitlicher das Crus I. Ventral davon liegt der Paraflocculus.

Caudal (Abb. 11 C) sehen wir die Beziehungen zwischen dem Lobulus medius und dem Crus II. Der Sulcus paramedianus ist in diesem Bereich zwischen Wurm und Kleinhirnhemisphären am tiefsten. Lateral von Crus II wird die kaudale Fläche des Crus I und des Paraflocculus sichtbar. Oberhalb des Fissura secunda liegt die Fissura praepyramidalis und zwischen beiden die Pyramis, die ebenfalls Läppchen zum Crus II entsendet.

Basal (Abb. 11 D) betrachtet sehen wir kranial den Lobulus centralis. Die

* Die hier verwendete Nomenklatur ist aus dem Werk von Nello Beccari: Neurologia comparata anatomo-funzionale dei vertebrati compreso l'uomo — Firenze 1943, entnommen.

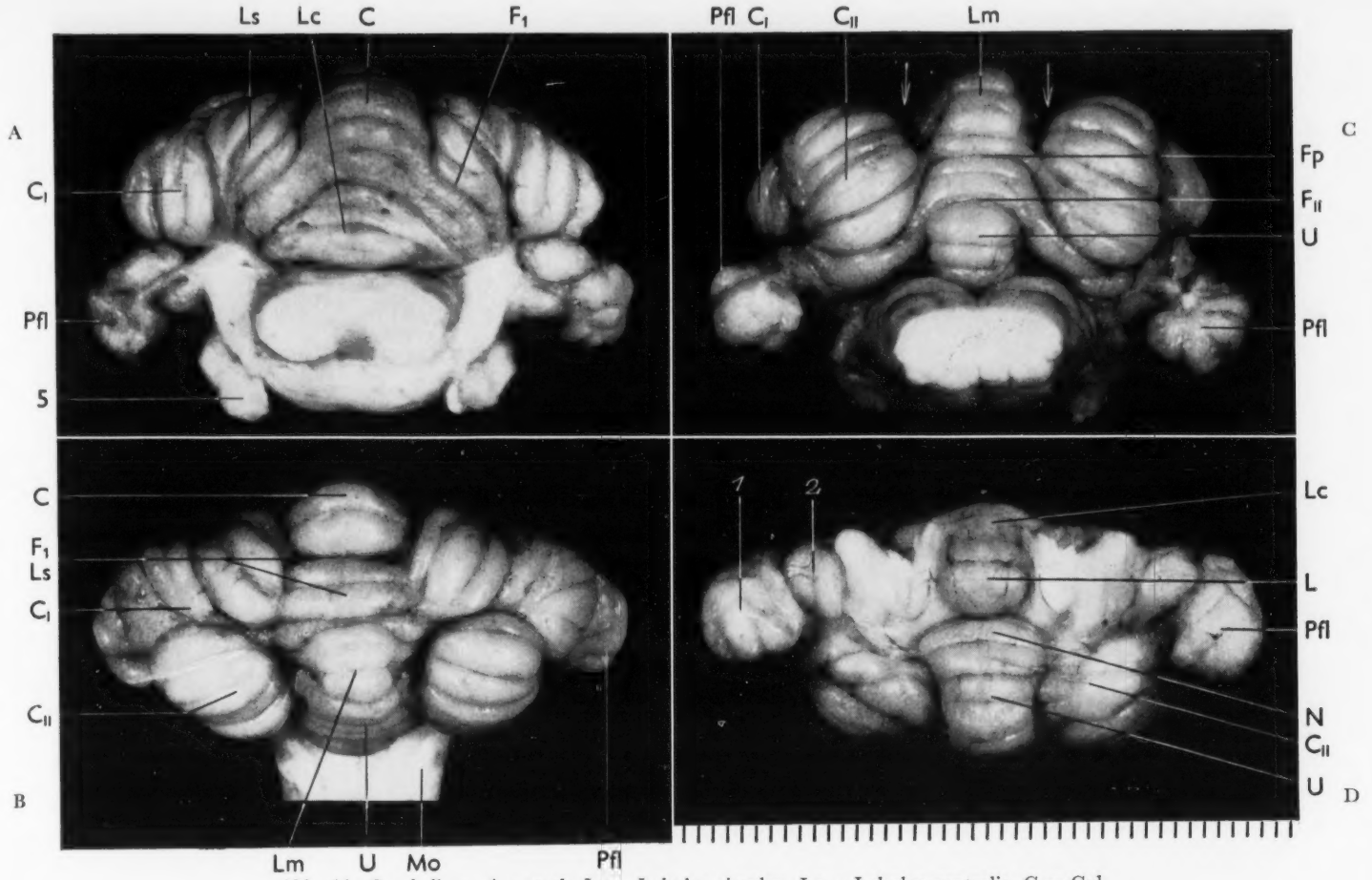

Abb. 11. *Cerebellum. A rostral:* Ls = Lobulus simplex, Lc = Lobulus centralis, C = Culmen, F_I = Fissura prima, C_I = Crus primum, Pfl = Parafloccculus, 5 = Nervus trigeminus. *B dorsal:* C = Culmen, F_I, Ls = Fissura prima und Lobulus simplex, C_I = Crus prinum, C_{II} = Crus secundum, Lm = Lobulus medius, U = Uvula, Mo = Medulla oblongata, Pfl = Parafloccculus. C caudal: Pfl = Parafloccculus, C_I C_{II} =Crus primum und secundum, Lm = Lobulus medius, ↓ = Sulcus paramedianus, Fp = Fissura praepyramidalis, F_{II} = Fissura secunda, U = Uvula. *D basal: Lc* = Lobulus centralis, L = Lingula, Pfl = Parafloccculus, N = Nodulus, C_{II} = Crus secundum, U = Uvula, 1 = Parafloccculus, 2 = Flocculus.

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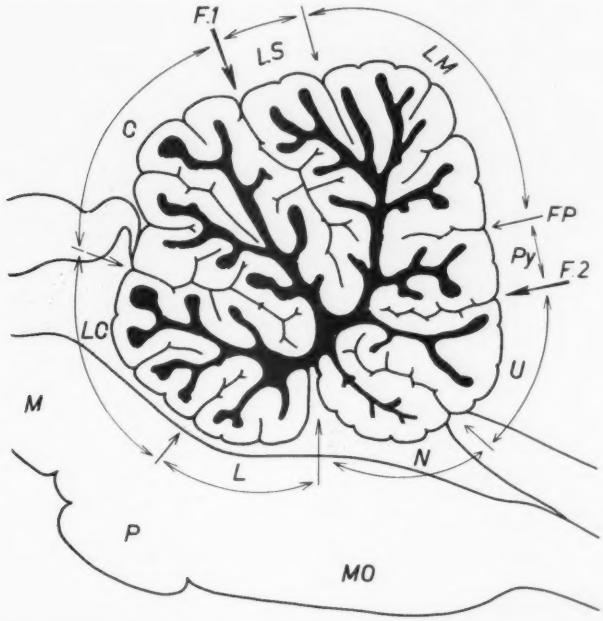

Abb. 12. *Mediansagittalschnitt durch das Kleinhirn* (Schematisch): M = Mittelhirn, P = Pons, MO = Medulla oblongata, L = Lingula, LC = Lobulus centralis, C = Culmen, F_1 = Fissura prima, LS = Lobulus simplex, LM = Lobulus medius, FP = Fissura praepyramidalis, Py = Pyramis, F_2 = Fissura secunda, U = Uvula, N = Nodulus.

Lingula berührt den Nodulus. Seitlich setzt sich der Nodulus mittels zwei langen Stielen in den Flocculus fort. Der Paraflocculus, die orale Fläche vom Crus II und die Uvula sind gut zu sehen.

Auf einem *Sagittal*schnitt, welcher schematisch in der Abb. 12 dargestellt ist, können wir die mediale Ausdehnung der Kleinhirnformationen verfolgen: Lingula, Lobulus centralis, Culmen, Fissura I, Lobulus simplex, Lobulus medius, Fissura praepyramidalis, Pyramis, Fissura II, Uvula und Nodulus. Lingula, Lobulus centralis und Nodulus bilden das Dach der Rautengrube. Die Fissura I ist auf diesem Schnitt leicht dorso-cranial gerichtet, die Fissura II ist horizontal kaudal orientiert.

Frontalschnitte

Die Frontalschnitte, die in den Abbildungen 13, 14, 15 dargestellt sind, stammen aus zwei Gehirnen (T 27 und T 28) und sind in topographischer Reihenfolge angeordnet.

A) Die Grosshirnhemisphäre hat in dieser Frontalhöhe eine mehr dreieckige

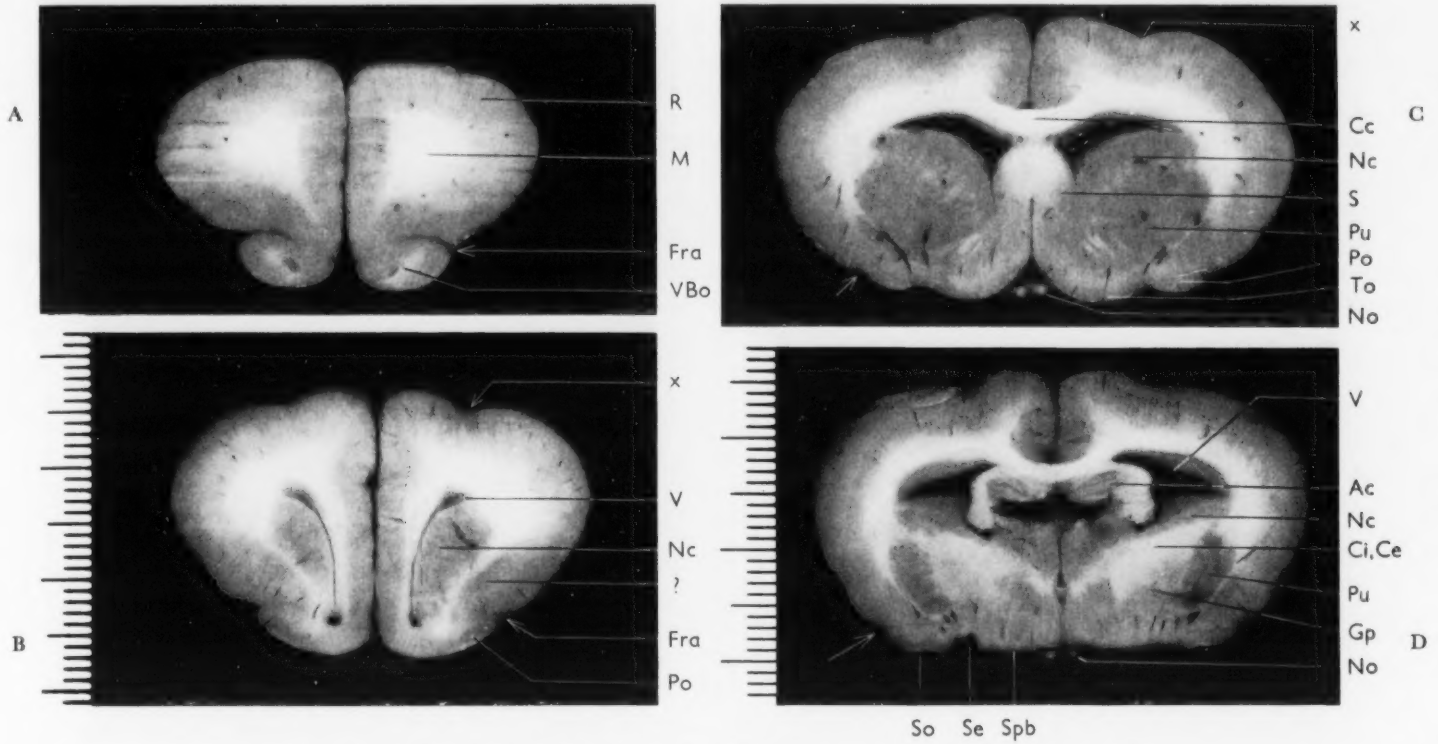

Abb. 13. *Frontalschnitt A:* R = Neocortex, M = Frontalmark, Fra = Fissura rhinalis anterior, V Bo = Ventrikel des Bulbus olfactorius. *Frontalschnitt B:* X = *Fissura parrasagittalis*, V = Ventrikel (Vorderhorn), Nc = Nucleus caudatus (Caput), ? = Claustrum(?), Fra = Fissura rhinalis anterior, Po = Pedunculus olfactorius, (zwischen Fra und Po der Gyrus olfactorius lateralis). *Frontalschnitt C:* X = Fissura parasagittalis, Cc = Corpus callosum, Nc = Nucleus caudatus, S = Septumgrau, Pu = Putamen, Po = Pedunclus olfactorius, To = Tuberculum olfactorium, No = Nervus opticus, ↑ = Palaeo-neocorticale Grenze (Fissura rhinalis). *Frontalschnitt D:* V = Seitenventrikel, Ac = Archicortex, Nc = Nucleus caudatus, Ci, Ce = Capsula interna und Capsula externa, Pu = Putamen, Gp = Globus pallidus, No = nervus opticus, Spb = Spatium parolfactorium basale, Se = Sulcus endorhinalis, So = Stria olfactoria, → = palaeo-neocorticale Grenze.

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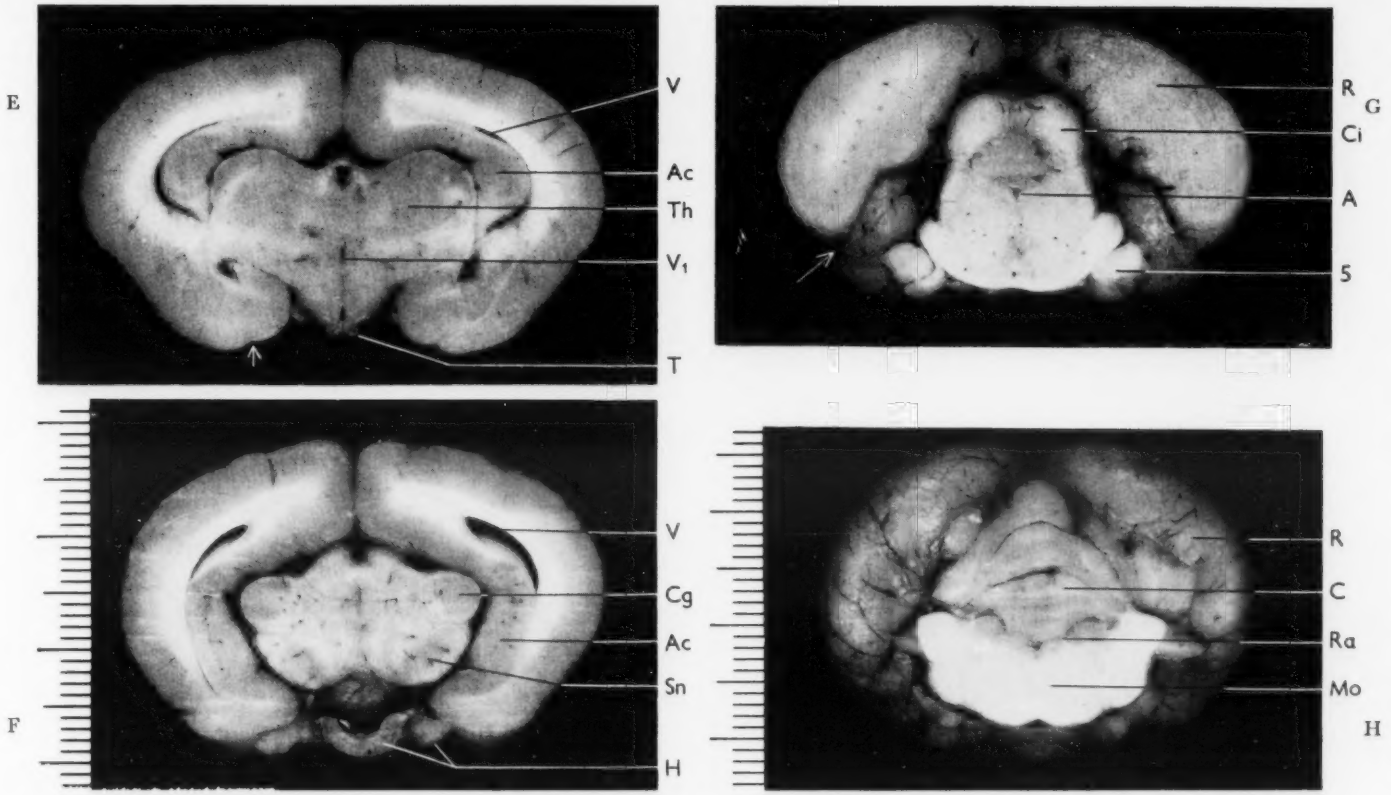

Abb. 14. *Frontalschnitt E:* V = Ventrikel, Ac = Archicortex, Th = Thalamus, V_1 = III. Ventrikel, T = Tuber, ↑ = palaeo-neocorticale Grenze. *Frontalschnitt F:* V = Ventrikel, Cg = Corpus geniculatum, Sn = Substantia nigra, Ac = Archicortex, H = Hypophyse und Nervus trigeminus. *Frontalschnitt G:* R = Neocortex, Ci = Colliculus inferior, A = Aquaeductus Sylvii (caudaler schmaler Abschnitt), 5 = Nervus trigeminus, → = palaeo-neocorticale Grenze. *Frontalschnitt H:* R = Neocortex (occipital), C = Kleinhirn, Ra = Rautengrube, Mo = Medulla oblongata.

Form; die tiefe Fissura rhinalis anterior und der Übergang des Palaeocortex in den Neocortex des Frontalhirns sind deutlich zu sehen. Die Rindenschicht der neocorticalen Gebiete ist, wie auch auf den nachfolgenden Schnitten sehr breit, was ein Charakteristikum des Nagetiergehirns ist. Im mittleren Bereich des Palaeocortex ist noch ein kleiner Abschnitt des Ventriculus olfactorius zu sehen.

B) Dorsal wird die parasagittal verlaufende Rindenfurche getroffen. Man sieht auf dem Frontalschnitt sehr deutlich, wie sich die gesamte Rindenschicht bis zum Mark um die Furche eindellt. Die Ventrikel sind hier spaltförmig und vertikal ausgedehnt mit einer dorsalen und oralen Erweiterung, zwischen denen sich der Kopf des Nucleus caudatus einschiebt, und sich medial vorwölbt. Eine Marklamelle grenzt das Grau des Schwanzkernes seitlich ab. Zwischen dieser und der noch tiefen Fissura rhinalis anterior erscheint eine graue Formation, deren Natur histologisch zu klären ist (sehr rostral gelegenes Claustrum, vordere Putamenabschnitte?). Zwischen Fissura rhinalis anterior und Pedunculus olfactorius verläuft der Gyrus olfactorius lateralis.

C) Dieser Schnitt ist hinter dem Balkenknie geführt. Ganz dorsal paramedian sieht man die Hemisphärenfurche gut. Dorsomedial, oberhalb des stark entwickelten Balkens, liegt die cinguläre Rinde. Die Ventrikel sind weit, entsprechend der lateralen Ausdehnung des Gehirnes mehr in horizontaler Richtung entfaltet. Oral vom Balken findet sich die Umschlagstelle des Fornix mit den entsprechenden grauen Formationen der Septumregion, die in das Tuberculum olfactorium kontinuierlich übergehen. Das Striatum wird hier in seiner maximalen Ausdehnung getroffen. Zwischen Putamen und Caudatum ist eine Capsula interna sichtbar. Oral von dieser findet sich die seitliche Aufsplitterung der Commissura anterior. Das Tuberculum olfactorium ist im mittleren Bereich getroffen. Seitlich vom Tuberculum verläuft der Sulcus endorhinalis. Zwischen ihm und der Fissura rhinalis finden sich die Formationen des Pedunculus olfactorius.

D) Der Schnitt ist knapp vor dem Chiasma, hinter dem Foramen Monroi geführt. Die Ventrikel sind weiter als auf dem vorherigen Querschnitt, etwa dreieckförmig. Medial, unter dem Balken, wird der oralste Abschnitt der Hippocampusformation mit dem Fornix getroffen, die dachförmig dem vorderen Thalamus aufliegen. Das Corpus nuclei caudati ist abgeplattet und bildet den Boden des Seitenventrikels. Die Capsula interna ist hier stärker ausgebildet. Medial vom Putamen tritt ein sehr gut entwickelter Globus pallidus auf. Der dritte Ventrikel ist spaltförmig und in ihm wird die Commissura anterior sichtbar. Baso-lateral sehen wir die Fissura rhinalis, das kaudale Ende des Sulcus endorhinalis, die Stria olfactoria und ganz medial das breite und flache Gebiet des Spatium parolfactorium basale und die Nervi optici.

E) Der Schnitt geht durch die Mitte der Pars oralis tuberis, hinter dem Balkensplenium. Der Seitenventrikel schmiegt sich an die Wölbung des Hippocampus an und wird durch dessen starke Ausbildung spaltförmig. Der Thalamus nimmt in seiner lateralen Ausdehnung mächtig zu. Um den dritten Ventrikel findet sich

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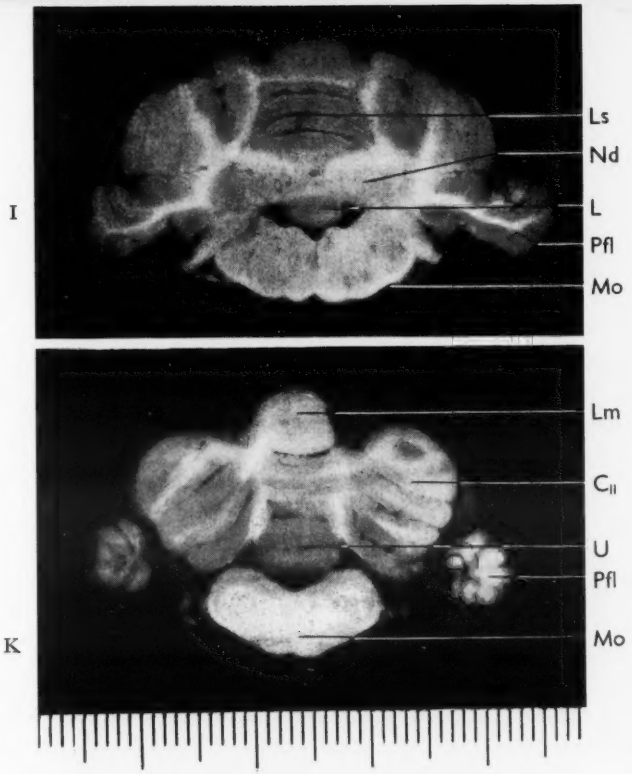
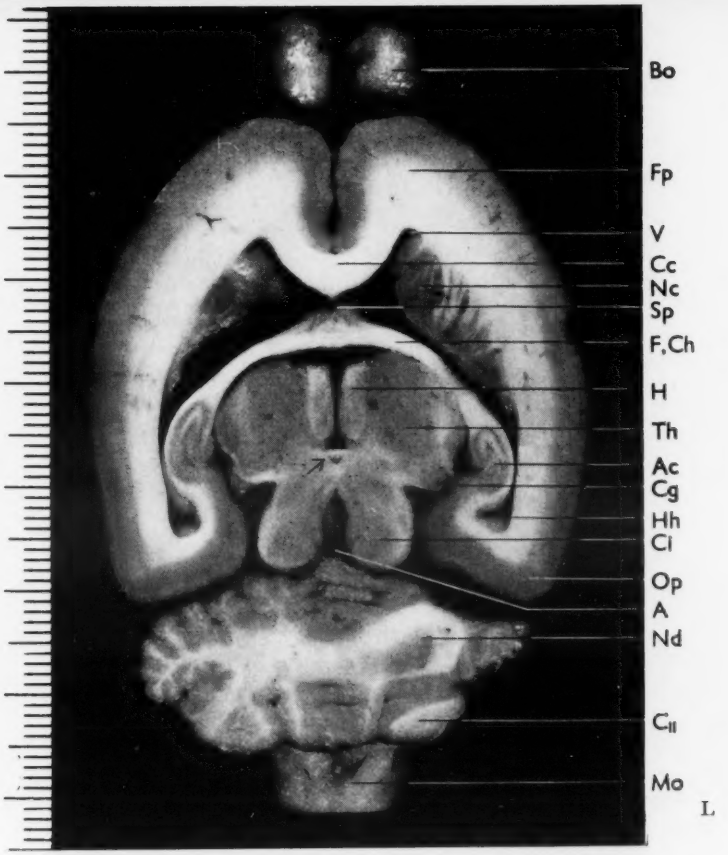


Abb. 15. *Frontalschnitt I:* Ls = Lobulus simplex, Nd = Nucleus dentatus, L = Lingula, Pfl = Paraflocculus, Mo = Medulla oblongata. *Frontalschnitt K:* Lm = Lobulus medius, C_{II} = Crus secundum, U = Uvula, Pfl = Paraflocculus, Mo = Medulla oblongata.



Horizontalschnitt L: Bo = Bulbus olfactorius, Fp = Frontalpol, V = Ventrikel, Nc = Nucleus caudatus, Cc = Corpus callosum, Sp = Septum pellucidum, F, Ch = Fornix und Commissura hippocampi, H = Habenula, Th = Thalamus, Ac = Archicortex, Cg = Corpus geniculatum, Hh = Hinterhorn des Ventrikels, Ci = Colliculus inferior, Op = Occipitalpol, A = Aquaeductus Sylvii, Nd = Nucleus dentatus, C_{II} = Crus secundum, Mo = Medulla oblongata, → = Commissura posterior.

das markarme Grau des Hypothalamus. Der oralste Abschnitt der Fissura intrapyriformis ist getroffen.

F) Der Frontalschnitt trifft die Hypophysenmitte und die Colliculi superiores der Vierhügelplatte. Die Seitenventrikel sind wieder weit geworden, der Archicortex ist mehr basal gelegen und umfasst die Formationen des Zwischen- und Mittelhirns, die auf diese Frontalebene zu liegen kommen: Corpus geniculatum (laterale ?), Mittelhirngrau und Pedunculus cerebri. Im Bereich des Pedunculus sieht man deutlich die Substantia nigra.

G) Der Schnitt geht durch die Colliculi inferiores der Lamina quadrigemina und den Pons. Die palaeo-neocorticalen Beziehungen sind durch die Fissura rhinalis posterior gezeichnet.

H) Die Verhältnisse sind denen des vorhergehenden Schnittes ähnlich; die Brücke ist durch die Brachia pontis ad cerebellum breiter geworden. Der Lobulus centralis des Kleinhirns ist getroffen.

I, K, L) Diese Schnitte betreffen nur das Kleinhirn und die Medulla oblongata, Strukturen auf die wir in der makroskopischen Darstellung des Bibergehirns nicht eingehen wollen. Sie werden Gegenstand mikroskopischer Untersuchungen sein. Die Grundformationen sind aus den Bildern ersichtlich.

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Horizontalschnitt

(Abb. 15 L)

Die Sektion ist knapp dorsal den Bulbi olfactorii geführt und trifft die Colliculi inferiores der Lamina quadrigemina. Der Balken ist rostral gelegen. Die Liquorräume lassen ein Vorderhorn und ein Hinterhorn erkennen. Das kurze, auf den Frontalschnitten nicht getroffene, Septum pellucidum spannt sich zwischen Balken und Commissura hippocampi. Die Habenulae sind mächtig enwickelt und markreich. Der Thalamus ist quergelagert und befindet sich in einer kaudal offenen Nische, die von der Commissura hippocampi und dem Archicortex gebildet wird.

Parasagittalschnitte

(Abb. 16)

Deutlicher sind auf diesen Aufnahmen die Beziehungen zwischen Palaeo-, Neo- und Archicortex mit deren Übergangszonen und Furchen, sowie die topographischen Verhältnisse zwischen Hippocampus und Thalamus zu sehen.

Abb. 16. *Parasagittalschnitte:* R = Neocortex, Ac = Archicortex, V = Seitenventrikel, Nc = Nucleus caudatus, Pu = Putamen, ? = Claustrum(?), Fra = Fissura rhinalis anterior, Po = Pedunculus olfactorius, Cg = Corpus geniculatum, Th = Thalamus, Pco = Palaeocortex, Ci = Capsula interna, Frp = Fissura rhinalis posterior.

Tabelle 5

SNr.	T 21	T 22	T 23	T 24	T 26	T 27	T 28	T 32
Länge des Grosshirns	49	50	48	49	45	47	48	42
Breite des Grosshirns	45	47	45	50	43	45	48	46
Höhe des Grosshirns	30	30	28	31	28	30	—	30
Horizontaler Umfang des Grosshirns	160	—	165	180	160	170	180	165
Frontaler Umfang des Grosshirns	130	—	127	130	120	130	130	125
Länge des Kleinhirns	22	20	19	21	20	20	21	22
Breite des Kleinhirns mit Paraflocculi	46	46	43	—	40	40	46	40
Breite des Kleinhirns ohne Paraflocculi	36	36	33	38	33	35	36	—
Höhe des Paraflocculus	7	8	7	—	7,5	9	8	—
Höhe des Kleinhirns mit Brücke	25	—	25	28	24	26	26	26
Länge der Brücke	7,5	7,5	6	6,5	6,4	—	—	7
Breite der Brücke	—	23	22	23	20	21	21	22
Länge des Bulbus olfactorius	17	16	18	18,5	15	15,5	17	16,5
Breite des Bulbus olfactorius	6	7	6	7,5	6	7	7,5	6,5
Länge des Tractus olfactorius	15	17	16	15	14	14,5	20	16
Breite des Tuberculum olfactorium	9	10	9	11	8,5	9	9	9,5
Entfernung zwischen den Fissurae rhinales	35	37	35	38	33	35	36	35
Fissura rhinalis → Uncus pyriformis	13	—	13	14,4	13	13	13	—
Kleinste Enterfernung zwischen den Unci pyriformes	9	10,5	10	11	9	10	10	11,5
Länge des Hypothalamus	10	10	10	11	10	10,5	10,3	10
Durchmesser des Nervus opticus	1	1	—	—	—	—	—	—
Durchmesser des Nervus trigeminus	5	—	—	6,4	—	—	—	6
Durchmesser des Nervus facialis	—	—	—	—	—	—	1,5	—
Durchmesser des Nervus acusticus	—	—	2	—	—	—	2,5	—
Länge des Balkens	—	17,5	—	—	14	—	—	—
Balkenknie → Frontalpol	—	14	—	—	14	—	—	—
Balkenmitte → Mantelkante	—	9	—	—	7	—	—	—
Balkensplenium → Occipitalpol	—	27	—	—	26	—	—	—
Länge der Massa intermedia		9,5	—	—	10,1	—	—	—
Höhe der Massa intermedia	—	9	—	—	7,5	—	—	—
Mitte der Massa intermedia → Infundibulum	—	11	—	—	9	—	—	—
Länge des Corpus mammillare	—	3	—	—	3,6	—	—	—
Länge der Lamina quadrigemina	—	11	—	—	10	—	—	—
Länge der Epiphyse	—	3	—	—	3	—	—	—
$\frac{\text{Hypothalmuslänge}}{\text{Grosshirnlänge}}$	0,20	0,20	0,21	0,22	0,22	0,22	0,21	0,24
Hirngewicht gr	44	53	45	52	38	43	48	43
Hirnvolumen ccm	45	55	45	55	37	45	48	40

N.B.: Sämtliche Streckenmasse in mm.

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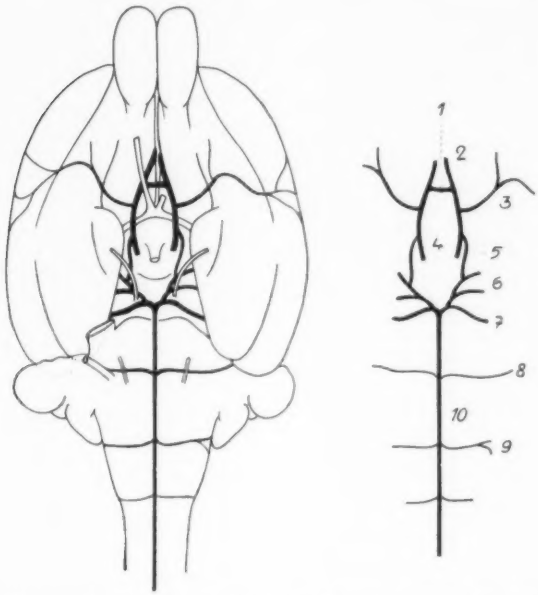

Abb. 17. *Basale Hirnarterien* (schematisch): 1 = Ramus communicans anterior, 2 = Art. cerebri anterior, 3 = Art. cerebri media, 4 = Art. carotis interna, 5 = Ramus communicans posterior, 6 = Art. cerebri posterior, 7 = Art. cerebelli superior, 8 = Art. cerebelli inferior anterior, 9 = Art. cerebelli inferior posterior, 10 = Art. basilaris.

Gefässapparat

Die arterielle Versorgung des Gehirns ist schematisch in der Abb. 17 wiedergegeben. Der Verlauf des Circulus Willisii ist von dem der übrigen beschriebenen Säuger nicht sehr abweichend. Die Zeichnung erübrigt eine spezielle Beschreibung. Für eine Darstellung der venösen Bluträume des Gehirnes war unser Material ungenügend.

Hirnmessungen

Auf der Tabelle 5 bringen wir eine detaillierte Zusammenstellung sämtlicher Messungen, die am Gehirn nach etwa 3 Wochen Formolfixation durchgeführt wurden.

Schädel

Zum Zwecke der Systematik und der craniocerebralen Beziehungen wurden Schädel von erwachsenen Männchen und Weibchen gemessen und davon Endocranialausgüsse hergestellt. Der Schädel des kanadischen Bibers (Tab. 6 a, 6 b)

ist, wie eigene Messungen zeigen, im wesentlichen kürzer als der der europäischen Art, welcher ausserdem massiver gebaut erscheint (Tab. 7 a, 7 b). Ob auch das Gehirn beider Arten Unterschiede aufweist, können wir in Ermangelung des europäischen Materials vorläufig nicht feststellen.

Tabelle 6 a

SNr.	T 25 ♀	T 57 ♂
Schädellänge	138	139
Schädelbreite	103	98
Schädellänge zu Schädelbreite in %	74,6	70,5
Nalsalia-ıänge	46	51
Nasalia-breite	14	15
Schädellänge zu Nasalia-länge in %	33,3	36,7
Länge der Crista sagittalis	21	31
Länge des knöchernen Gehörgangs	15	14
Diastema Oberkiefer	54	46
Länge des Unterkiefers	124	127
Breite des Unterkiefers	91	102
Diastema Unterkiefer	23	25

N.B.: Masse in mm.

Tabelle 6 b

SNr.		Oberkiefer Kaufläche Länge × Breite		Unterkiefer Kaufläche Länge × Breite	
		li	re	li	re
T 25	Schneidezahn	9,0×7,5	10,0×7,5	17,4×8,5	17,0×8,4
	Praemolar	10,0×9,0	10,0×9,5	10,9×7,3	8,0×7,7
	1. Molar	8,0×7,5	8,0×8,3	8,0×7,8	8,4×8,5
	2. Molar	6,0×7,0	6,3×7,4	8,5×7,5	9,8×7,8
	3. Molar	5,9×6,5	6,0×7,7	9,0×6,4	9,6×6,7
T 57	Schneidezahn	11,0×7,8	12,0×7,5	19,0×8,0	20,0×8,0
	Praemolar	10,5×8,5	9,3×8,4	12,3×10,0	12,0×10,0
	1. Molar	7,5×8,3	8,3×8,0	9,0×10,1	8,5×10,3
	2. Molar	7,0×7,5	7,0×6,3	9,0×9,6	9,0×9,5
	3. Molar	7,0×7,0	6,0×6,5	9,0×8,0	9,0×8,0

Länge der Schneidezähne

SNr.	Oberkiefer		Unterkiefer	
	li	re	li	re
T 25	90	91	128	140
T 57	95	94	139	124

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Tabelle 7 a

Castor Fiber LIN.*

SNr., Tierangaben	"Elbe-biber, Dr. Bieder-mann 1918"	573	"♂-Zoo Basel 7.7.39"	5762	"♀ Elbe-biber, Max Planck Institut-Giessen"
Schädellänge	150	155	143	150	167
Schädelbreite	98	104	98	96	105
Schädellänge zu Schädelbreite in %	65,3	67,1	68,5	64,0	62,9
Nasalia-länge	63	63	60	58	63
Nasalia-breite	16	17	14	16	17
Schädellänge zu Nasalia-länge in %	42,0	40,6	42,0	38,7	27,0
Länge der Crista sagittalis	28	32	25	24	39
Länge des knöchernen Gehörgangs	15	16	13	15	18
Diastema Oberkiefer	47	51	46	47	51
Länge des Unterkiefers	120	132	120	120	138
Breite des Unterkiefers	85	95	83	90	90
Diastema Unterkiefer	28	27	24	25	28

N.B.: Masse in mm.

* Die Überlassung des Materials verdanken wir Herrn Dr. Künzi, Direktor des Naturhistorischen Museums in Bern.

Tabelle 7 b

SNr., Tierangaben		Oberkiefer Kaufläche Länge × Breite		Unterkiefer Kaufläche Länge × Breite	
		li	re	li	re
"Elbebiber, Dr. Biedermann 1918"	Schneidezahn	—	8,0×7,0	–	12,0×7,0
	Praemolar	9,0×8,0	9,5×8,5	10,5×8,0	10,0×8,5
	1. Molar	7,0×8,0	7,0×7,0	8,0×8,0	8,0×8,5
	2. Molar	7,0×9,0	7,0×7,0	8,0×8,0	8,5×7,7
	3. Molar	6,5×7,0	7,0×7,0	8,0×7,2	9,0×6,5
573	Schneidezahn	11,5×9,0	11,0×9,0	11,7×9,0	12,0×9,5
	Praemolar	10,0×9,0	9,0×9,0	11,0×8,0	11,0×9,0
	1. Molar	8,0×8,5	8,0×8,5	—	9,0×9,0
	2. Molar	7,0×8,0	7,0×8,4	—	8,5×8,5
	3. Molar	7,2×7,5	7,0×8,0	8,0×7,4	7,0×7,0
"♂-Zoo Basel 7.7.39"	Schneidezahn	11,0×9,0	10,0×9,0	12,5×8,5	13,0×9,0
5762	Schneidezahn	–	11,0×8,0	—	10,0×8,0
	Praemolar	9,5×7,5	10,0×7,5	10,5×7,5	10,0×8,0
	1. Molar	7,0×8,0	7,0×7,0	8,0×8,0	8,0×8,0
	2. Molar	7,0×7,5	7,0×7,0	7,5×7,5	8,2×7,5
	3. Molar	6,7×7,0	7,0×6,7	7,5×7,0	7,0×6,5
"♀ Elbebiber, Max Planck Institut, Giessen"	Schneidezehn	11,5×9,0	12,0×9,0	15,0×9,0	15,0×9,0
	Praemolar	10,0×9,5	11,0×9,0	10,0×8,0	10,0×8,0
	1. Molar	8,0×9,5	8,0×9,5	8,0×9,5	9,0×9,0
	2. Molar	7,0×9,0	7,0×8,5	9,0×9,0	8,5×9,0
	3. Molar	7,0×8,0	6,5×8,0	8,2×8,0	8,0×7,5

G. Pilleri

Cranio-cerebrale Topographie

Es ist ein grosser Verdienst von H. Spatz, die Aufmerksamkeit der vergleichend-neurologischen Forschung und der Anthropologie auf die Morphologie des *Endocranium* gelenkt zu haben.

Betrachtet man den Schädel eines erwachsenen Bibers, so fällt sofort ein für die Wassersäuger* charakteristisches Anpassungssystem auf (O. Veit): Im Endocranium ist das Cavum cerebellare (seu rhombencephalicum) durch kein Tentorium osseum vom Cavum cerebrale getrennt. Eine unregelmässig verlaufende Crista petrosa, an der das Tentorium cerebelli ansetzt, zeichnet die Grenze zwischen beiden Schädelbereichen. Sie reicht bis zur Spitze der Felsenbeinpyramide. Im Cavum cerebrale (Abb. 18) vermisst man die Einteilung in zwei Schädelgruben, eine vordere und eine mittlere, fast ganz. Der caudale Flügelrand des Praesphenoid verliert sich in ein Gebiet, das stark von mehreren Furchen der Arteria duralis meningica media gefurcht erscheint. Hier weist das Cavum cerebrale seine grösste Querausdehnung auf. Das Praesphenoid, Basissphenoid und basioccipitale sind im mittleren Bereich fast gleich lang. Auffallend ist beim Biberschädel die Länge des medialen Abschnittes der mittleren Schädelgrube (Nervengefässfach). Die Schädelbasismitte (Distanz zwischen Synchondrosos intersphenoidalis und Synchondrosis sphenoocipitalis) misst 20 mm (T 25) und 23 mm (T 57). Die Länge des Cavum cerebrale (Distanz zwischen Jugum limitans fossae olfactoriae und Crista petrosa, laterales Ende) beträgt 47 mm (T 25) und 51 mm (T 57). Der Index der Schädelbasismitte (Spatz, Schuchardt) ist

$$\frac{20 \times 100}{47} = 42{,}6 \quad (\text{T } 25)$$

und

$$\frac{23 \times 100}{51} = 45{,}1 \quad (\text{T } 57)$$

Rostral endet das Cavum cerebrale mit der tiefen und hohen Riechgrube (= Fossa olfactoria, Bulbuskammer). Die Siebbeinplatte liegt vertikal. Die Crista galli ist gut ausgebildet. Der Eingang der Bulbuskammer ist beidseits von einer kräftigen Knochenleiste (Jugum limitans fossae olfactoriae) flankiert, welche basal und dorsal fehlt. Sie zeichnet die scharfe Grenze zwischen Bulbus und Tractus olfactorius. Von der Mitte der Leiste geht nach kaudal das Jugum rhinicum orale. Es begrenzt eine schräge orale Aushöhlung, welcher der Pedunculus olfactorius aufliegt. Aboral vom Jugum rhinicum findet etwas frontaler Neocortex Platz. Das Jugum rhinicum orale hat einen kurzen Verlauf und verschwindet vor den Sulci der Arteria meningica media. Von der Synchondrosis intersphenoidalis geht eine mediale Rinne rostralwärts auf dem Planum praesphenoideum. Sie entspricht dem fast parallelen Verlauf der Nervi optici, die erst im Knochenkanal zu den

* Eine Ausnahme stellen erwachsene Delphine (*Tursious truncatus*) und *Phoca* dar, bei denen wir häufig ein verknöchertes Tentorium feststellen konnten.

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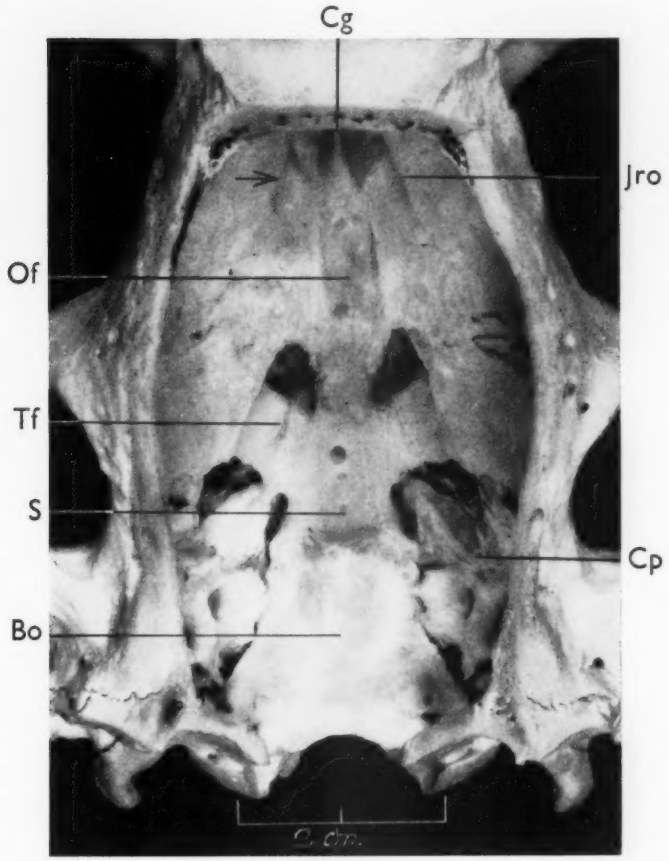

Abb. 18. *Basales Endocranium:* Cg = Crista galli, Jro = Jugum rhinicum orale, Of = Nervus opticus — Fach, Tf = Trigeminusfach, S = Sella, Bo = Basioccipitale, Cp = Crista petrosa.

Augen divergieren. Das Tuberculum olfactorium imprimiert sich nicht auf das Endocranium. Die Sella ist längsgezogen und sehr schwach ausgehöhlt. Gegen die Synchondrosis sphenooccipitalis findet sich die Andeutung eines Dorsum sellae. Ein Tuberculum sellae ist nicht entwickelt. Das Nervengefässfach wird fast ganz von einer breiten flachen Rinne eingenommen, worauf der Trigeminus liegt. Seitlich davon ist das Temporalhirnfach (das bei den meisten Rodentia dem Lobus pyriformis entspricht) glatt. Die palaeoneocorticale Grenze (SPATZ) wird hier durch *kein* Jugum gezeichnet (Jugum rhinicum aborale). Lediglich die feine Sutura sphenosquamosa, die latero-basalwärts verläuft, zeigt womöglich die topographische endocraniale Grenze zwischen Palaeo- und Neocortex, die am Gehirn durch die Fissura rhinalis posterior gegeben wird. An der endocranialen Fläche

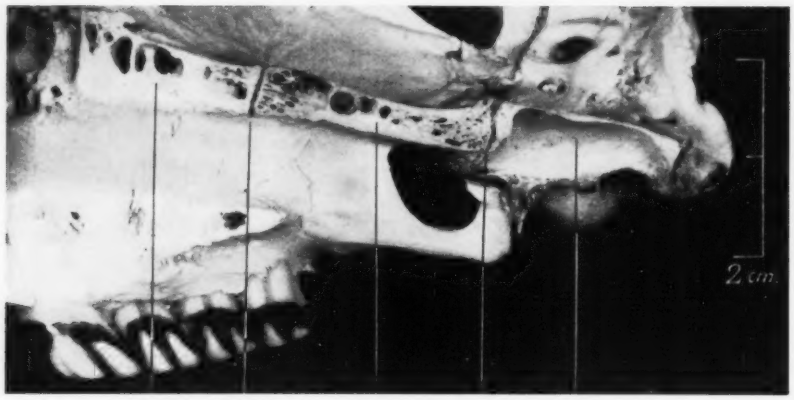

Abb. 19. *Mediansagittalschnitt durch die Schädelbasis:* Bo = Basioccipitale, Sso = Synchondrosis spheno-occipitalis, Bs = Basissphenoid, Sis = Synchondrosis intersphenoidalis, Ps = Praesphenoid.

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des Schädeldaches finden sich im vorderen Bereich der Parietalia 2 parasagittal verlaufende, kräftige und breite Juga, denen an der Oberfläche der Grosshirnhemisphären die zwei von LEURET und GRATIOLET und uns beschriebenen Längsfurchen entsprechen. In der übrigen Kalotte ist die Endocranialwand stark von Gefässfurchen durchzogen.

Ergebnisse

Betrachten wir die makroskopische Untersuchung des Gehirns von *Castor canadensis,* so kommen wir auf folgende Schlussfolgerungen:

1. Das Verhältnis Hirngewicht (= 1) zum Körpergewicht ist gleich 1 : 330. Der Biber ist *niedrig cephalisiert,* vergleicht man dieses Verhältnis mit dem anderer *Sciuromorpha* (z. B. *Sciurus vulgaris,* LIN.: 1 : 35 bis 1 : 60 — nach WEBER).
2. Der von GRÜNTAHL eingeführte Index Hypthalamuslänge: Grosshirnlänge, als Ausdruck der phylogenetischen Rangordnung eines Tieres, liegt beim Biber zwischen 0,20 und 0,24, was für ein hochentwickeltes Gehirn *im Rahmen der Nagetiere* sprechen würde.
3. Die Ausdehnung des Bulbus, Tractus olfactorius und der übrigen zum Rhinencephalon gehörigen Formationen (Lobus pyriformis, Habenula, usw.) lassen beim Bibergehirn, trotz vorzüglicher Wasseranpassung, das zentralnervöse Organ des *Makrosmatikers* erkennen. Vergegenwärtigen wir uns die Dimensionen der Ethmo-turbinalia im Nasenraum, der Drüsen, welche Richstoffe in die Kloake sezernieren (Geilsäcke) und die Menge solcher Substanzen in

den Drüsenlichtungen, erscheint uns die Entwicklung des Rhinencephalon eine durchaus entsprechende. Es wäre nicht ausgeschlossen, dass die Nasenrezeptoren des Bibers sogar unter Wasser funktionieren könnten.

4. Der *Neocortex ist sehr ausgedehnt* mit Bildung von Polen und Stauchungen, die an Hand des Winkelmessverfahrens von SCHNEIDER am besten anschaulich werden. Die Fissura rhinalis (palaeo-neocorticale Grenze) liegt latero-basal; sie ist also nur bei lateraler und basaler Betrachtung sichtbar. Die quantitativen Verhältnisse zwischen Palaeo-, Neo- und Archicortex wollen wir im histologischen Teil unserer Arbeit feststellen. Die Hirnrinde ist sehr schwach gyriert. Die Gehirne von Paca, Capybara, Viscacia und anderer *Hystricomorpha* sind viel stärker gyriert als das Bibergehirn.
5. Die Ventrikel sind relativ weit. Was dies bedeutet, kann vielleicht die Kenntnis der Ventrikelverhältnisse bei einer grossen Zahl von Nagerarten erbringen. Ein Hinterhorn ist ausgebildet.
6. Das Endocranium wird an der *Kalotte durch die Konvexitätsrinde imprimiert*. Dies steht wahrscheinlich in Beziehung zur Hirnevolution und zur ontogenischen Hirnentwicklung. Hier hat die Rinde ihre Impressionsfähigkeit noch nicht eingebüsst. Wie SPATZ sagt, ist sie noch in Entwicklung begriffen. „Die Impressionsfähigkeit von Hirnteilen an der Wand des Endocraniums ist ein Indikator für eine diesen Hirnteilen auf der betreffenden Differenzierungsstufe eigene Ausdehnungstendenz (Propulsivität)" (SPATZ). Die Anwendbarkeit dieses vor allem am menschlichen Schädel entwickelten Gesetzes auf das Gehirn der Nagetiere wollen wir später überprüfen.
7. Was die übrigen Sinnesorgane betrifft, erscheint der *Nervus opticus* entsprechend der Augengrösse *schmal*. Der Nervus acusticus und die übrigen Hirnnerven sind unauffällig. Der Trigeminus ist besonders mächtig und nimmt mit seinem Verlauf den grössten Teil des Nervengefässfaches im Endocranium ein.
8. Die Hypophyse, besonders die *Adenohypophyse* ist mächtig enwickelt und berührt den freien Brückenrand. Die Pars oralis tuberis des Hypothalamus ist lang. Das Corpus mammillare ist sehr markreich.
9. Das Kleinhirn, ein der Gesamtmotorik übergeordnetes Organ, erscheint besonders im Hemisphärenteil gut entwickelt. Diese Ausdehnung geht parallel derjenigen der extrapyramidalen Ganglien. Wieviel vom Kleinhirn dem neocerebellaren Teil (im Sinne von BRODAL) zuzurechnen ist, bleibt noch zu untersuchen. Die Paraflocculi sind sehr gut entwickelt.
10. Der Balken ist in seiner Längsausdehnung *kurz* und überdeckt den Thalamus *nicht* vollständig. Die extrapyramidalen Ganglien (Putamen, Pallidum, Nucleus caudatus, Substantia nigra) sind, bei makroskopischer Betrachtung, gut entwickelt.

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Studies on Homologous Inhibition in the Chick Embryo

by

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Introduction

INDUCTION

Physiologic embryology deals with the causes underlying the appearance of new molecules and the formation of tissues and organs in the embryo. Scientists have long focused their attention on these problems. Many of them, like SPEMAN and MANGOLD, have concentrated mainly on the problem of induction, which is closely associated with the matters dealt with in the present investigation. Despite the great importance of this conception, it is exceedingly hard to define. In the literature, one is, in fact, often confronted merely by a description. This indicates

that the phenomenon denoted as induction is not yet sufficiently elucidated, although more than 25 years' research has been devoted to it.

Two definitions can be cited: they are given by HOLTFRETER & HAMBURGER in the chapter on Amphibians in the *Analysis of Development* (18). Firstly (p. 245): "A process which determines primarily the cytological fate of the reacting cells." Secondly (p. 275): "Unlike hormones, inductive stimuli operate only at certain stages, as a rule during early development, and they are normally ineffective unless there is intimate contact between inducing and reacting tissues." The first definition is the more interesting, and stresses the fact that there is a primary mechanism which directly affects differentiation. It also emphasizes the state of receptivity of the cells which are the object of induction.

The second definition is less general and is open to criticism, since—while pointing out the difference between inductors and hormones—it perhaps underlines too strongly the importance of contact between the inductor and the cells that are influenced by it. It should, however, be noted that the authors express themselves very cautiously, and that they later mention the work of NIU & TWITTY (1953), who demonstrated that the inductors are diffusible, and can operate at a distance.

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NATURE OF THE INDUCTORS

After the question of the existence of induction, the problem of the nature of the inductors arises. Many workers have tried to solve this problem, but the results have not been conclusive. Thus, extracts of highly varying origin have proved to induce the same tissue or organ; no correlation exists between the origin of an organ extract and the organ which it induces. The most divergent chemical products have been found to possess inductive qualities (see *e.g. Analysis of Development* 1955, p. 270).

Special mention can, however, be made of some experiments, of which the results are of paramount importance. HOLTFRETER (1934) put forward the hypothesis that differentiation is caused by one or several inductors. CHUANG (1939) and TOIVONEN (1940), using the so-called sandwich technique (in which the extract is placed between two pieces of ectoderm taken from a blastocoel), and introduction of tissue extracts into a blastocoel, showed the existence of at least two inductive factors, one "neuralizing" and the other "mesodermizing". The former proved to be stable, and the latter unstable. The hypothesis put forward by HOLTFRETER has also been confirmed by LEHMANN (1950), NIU & TWITTY (1953) and YAMADA & TAKATA (1956). (14, 15, 4, 44, 45, 22, 29, 58.)

The nature of natural inductors seems to have been somewhat clarified in recent years by physico-chemical analyses of the extracts used. In 1943, BRACHET obtained neural induction with the use of microsomic fractions of extracts from the liver and kidney, and in 1953 KUUSI tested several extracts of a nucleoprotein nature (1, 20). Unfortunately, the results were not always clear, and sometimes

were even contradictory. The investigations of YAMADA & TAKATA, as well as those of HAYASHI (1956), seem to indicate that the most active fractions of the organ extracts are those containing pentose nucleoproteins. NIU's studies (1956), using very precise techniques, showed that the nucleoproteins play a part in induction (58, 13, 27, see also 28).

To conclude this analysis, the following statement can be made. Whatever the chemical nature of the inductors may be, they can be interpreted as being responsible for the appearance of new types of tissues. They do this by creating new tools, *e.g.* templates essential for further specialization of the cells in certain directions.

INHIBITORS

If the problem of induction has now become somewhat clarified by the work done at various laboratories, this did not apply some ten years ago. At that time, two American embryologists—WEISS and ROSE—formulated new theories regarding growth and differentiation. These theories are fairly similar. They postulate the existence—in addition to that of inductive and growth-promoting factors—of inhibitory ones, the balance of these factors then influencing the formation of new tissues, as well as the balance of their masses in the adult organism (51, 31, 32).

In 1947, WEISS put forward a theory concerning the specific control of growth, which he later summarized in the following way. Protoplasm synthesis of a given organ yields (a) templates for further reproduction, and (b) accessory diffusible compounds, capable of inactivating the former. As the latter, accumulating in the common humoral pool, reach critical concentration, growth ceases. Partial removal of an organ, by reducing the concentration of (b), will automatically entail "compensatory growth" in the rest of the organ.

WEISS and his co-workers have introduced these inhibitors of growth in a theory of differentiation. They created the conceptions of "templates" and "antitemplates", these denoting, on one hand, the positive factors of growth, acting as centres of protein synthesis and, on the other hand, the negative factors, inhibitors of growth, which appear as the tissues grow. According to WEISS, these "antitemplates" influence the "templates", neutralize their action, and a balance ensues which permits the tissues and organs to adjust in relation to each other (WEISS & KAVANAU 1957). (56.)

In 1952, ROSE published a theory on differentiation in an article entitled *A hierachy of self-limiting reactions as the basis of cellular differentiation and growth control.* He explained the problem of induction as follows. It is no longer a question of seeking the factors of induction of differentiation, but those factors must be sought which act in a negative way, *i.e.*, the homologous inhibitors. Inhibiting substances of growth appear in the different tissues of the organism in the course of their differentiation and growth. These substances are specific to each

tissue or organ; they act in an elective way on the formation of a larger quantity of the same tissue or organ. They are responsible for the balance of the amount of different organs, evading possible hypertrophy of any one of them.

These inhibiting factors do not act only in the adult organism. They are formed in the course of specialization of the different tissues, and may play an important role in differentiation of the embryo, by directing specialization of groups of cells towards formation of tissues not yet existing or little developed.

In a recent article, ROSE (1957) has again taken up the problem of differentiation through homologous inhibition. He gives a survey of the results of studies on the problem of interaction of cells during differentiation. In this review (p. 375), he makes a distinction between the inhibitors of growth which appear in the first stage of development of the egg, and are responsible for differentiation, and the inhibitors of growth which, in the adult, are responsible for the balance in the relative size of the organs. He shows that many results in experimental embryology, which are difficult to understand as applied only to the theory of induction, have their place within the theory of homologous inhibition. ROSE's *Cellular interaction during differentiation* (1957), and a complement to this review, *Failure of self-inhibition in tumor* (1958), make it superfluous to give a systematic review of these facts here (34, 35).

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The following conclusion can be drawn from the aforegoing review. Specialization under the influence of neighbouring tissues may include two complementary mechanisms, acting in different ways, *i.e.,* inductors creating new tools for further specialization of the cells, and inhibitors restricting the number of tools already existing in the cells.

There is, however, as yet little experimental foundation for the theory of homologous inhibition. In fact, the only experiments that seem to have been made are those of WEISS (53) and ROSE (33), *cf.* some results of SPRATT (43) in the chick or in Amphibians.

The object of the present investigation, started three years ago, was therefore to prove the validity of this theory by means of a large series of experiments, amenable to statistical analysis. The manifestation of the mechanism of homologous inhibition was studied at the beginning of the development of the chick embryo. Studies on the nature of the inhibitors are now in progress. Some preliminary results of this part of the investigation will reported on pp. 172—179 (*cf.* Discussion, pp. 189—191).

Material and Methods

I. HOST

Species

The hen's egg (white leghorn) was used for the experiments. This material has the following advantages:

(a) The normal development of the animal is known, and tables of reference are found in the manuals (6, 23).

(b) The white of the egg contains an antiseptic substance, which greatly diminishes the risks of infection.

(c) The blastoderm has a large surface in relation to that of its whole mass, and the possibility of contact with the injected extract is great. This surface has no resistant membranes; this applies particularly to the side lying against the yolk. These conditions facilitate absorption of the injected substances.

(d) Up to the 4th day after the beginning of incubation, the embryo is transparent. One can clearly discern the cerebral vesicles, nose, eye, ear, spinal cord and somites, the heart and its chambers, the visceral arches and, finally, the blood islands, or the vascular system and circulating blood. It is therefore possible to study a large material, which would not be the case if use were made only of series of histologic sections.

The new-laid eggs are placed in the incubator as soon as possible, but storage overnight at +4° C does not damage the embryos.

Age

In order to determine at which stage during development of the egg it would be suitable to make the intervention, series of preliminary experiments were made, using eggs of different ages during the first day of incubation.

Injection of "neutral" products, such as Tyrode solution alone or combined with horse serum, had no influence on development, provided that the intervention was made after formation of the primitive streak. When injection was performed before this stage of development, it resulted in the formation of monsters, consisting in most cases of small "humps" of cells, accompanied by some blood islands. This indicated that the injection should be made after formation of the primitive streak.

When organ extracts (blood, brain, heart) were injected after the primitive streak had started to form, or at the moment of its regression, anomalies characteristic of each category of extract appeared.

When the injection was made after regression of the primitive streak, no definite effect could be noted.

Consequently, after the preliminary experiments, injection was consistently made at the moment of regression of the primitive streak, *i.e.*, at stages 5—6 according to LILLIE's classification (23): see Fig. 1.

Size of Population

In view of the irregularity of the results, the experiments had to be made on groups of at least 25 embryos, the results obtained in several such groups then being assembled. Each series comprised eggs that had reached stage 4 in LILLIE's classification, but had not yet reached stage 7. Since the rate of development

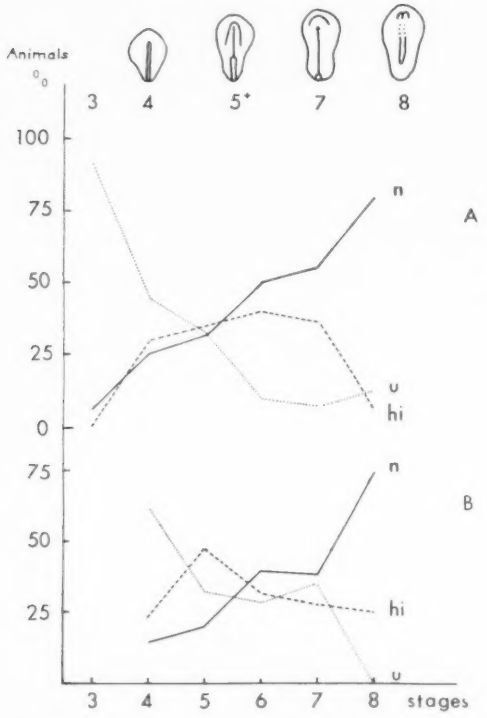

Fig. 1. Action of injection on eggs at different ages between stage 4 and stage 8. A. Brain extract. B. Blood extract. N = normal development. HI = homologous inhibition. U = formation of "humps".

showed large individual variations, the anatomic stage of the populations ranged from 4 to almost 7, with a maximum corresponding to stage 5—6 (Fig. 2).

Measure of Time

Owing to the individual variations in the rate of development, astronomic time cannot be used. It is necessary to use physiologic time, since this alone gives the exact age of the embryo both at the moment of injection and at the time of diagnosis. This age is based on anatomic criteria, *i.e.*, length of the primitive streak at the moment of injection (18—20 hours), and the stage of differentiation of the various organs and their parts at the time of diagnosis (4th day). In the present investigation, these criteria were based on the photographs in LILLIE's manual and the histologic sections reproduced in DUVAL's *Atlas d'Embryologie* (6).

II. PREPARATION OF EXTRACTS

At stages 5—7, the embryo is highly sensitive to variations in the pH and saline concentration of the milieu. All extracts for injection were therefore prepared

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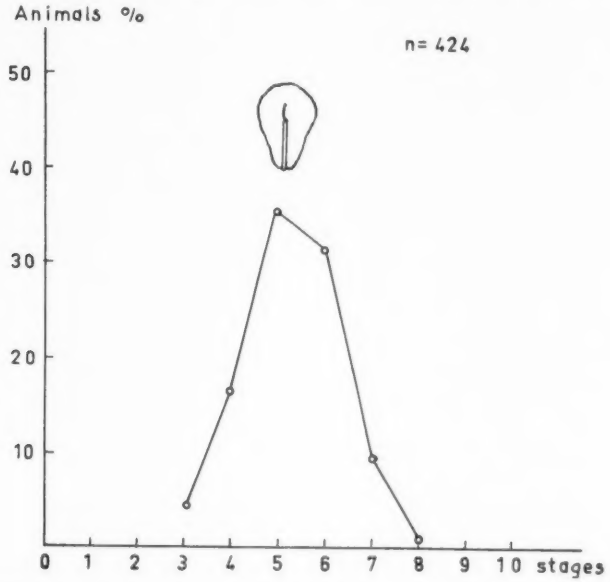

Fig. 2. Normal distribution of the developmental stages of the blastoderms, determined by comparison with LILLIE's table, at the moment of injection. n = number of embryos.

with isotonic Tyrode solution, and when necessary adjusted to pH 7.5. Preparation was always performed at 0—4° C, observing the usual precautions for prevention of infection.

The tissue selected for preparation of the extracts was taken from a healthy animal. It was carefully dissected, and all foreign tissue removed.

All traces of blood were removed from the tissues by washing in Tyrode solution, and the cardiac muscular tissue was freed from adherent fat and other foreign matter. The tissues were thoroughly homogenized with Tyrode solution in a Potter homogenizer for 15 minutes. The homogenate was allowed to stand for 24 hours at 0—4° C, and stirred occasionally. Sometimes the whole homogenate was used, but in most cases it was centrifuged at 3,000 r.p.m. for removal of the cell fragments. The supernatant was sterilized by passing through a sterile G5M Jena filter. In order to obtain an idea of the final concentration of the organ extracts for injection prepared in this way, the total N of an aliquot was determined by direct nesslerization of the digest, after combustion. The concentration was found to range from 600 to 1000 γ/ml.

In view of the technical difficulties encountered in preparation of blood, as well as in experiments with such preparations, it proved necessary in most cases to fractionate the blood. The following extracts were tested:

(1) *Sterile defibrinated whole blood,* diluted to 1 : 5 with Tyrode solution.

(2) *Red corpuscles.* The blood is centrifuged under sterile conditions, the red

corpuscles washed in Tyrode solution, and mixed with this fluid in a proportion of 1 : 5.

(3) *Cock serum.*

(4) *Hemolyzed red corpuscles.* The blood is hemolyzed by freezing, then diluted with Tyrode solution. The solution is filtered, and centrifuged at the same *g* and for the same time as in preparation of the microsomic fraction of the cells. The clear supernatant is sterilized by passing through a G5M Jena filter, and kept at +4° C.

(5) *Fractions of blood not precipitable by ammonium sulphate.* The blood is hemolyzed and, after filtration, the salt is added gradually and stirred. A gel appears, which is broken up by an excess of the salt, and yields a product which is separable by filtration. The object of this procedure is to remove the hemoglobin, which makes the experiments difficult, owing to its toxicity or that of its breakdown products. The sulphate is carefully eliminated by prolonged dialysis, first against running tap water (48 hrs), and then against distilled water (24 hrs). The core is lyophilized, and a white powder is obtained. This powder is dissolved in Tyrode solution and sterile-filtered (G5M Jena filter).

(6) *Horse serum.*

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III. INJECTION

From 25 to 30 newlaid eggs were incubated at 37° C for 18 to 20 hours. The egg-shell was washed with a 3 per cent alcoholic solution of iodine, and opened with sterile forceps. The injection needle was driven in at the margin of the germinative disc, and 0.06 ml of the relevant extract was slowly injected into the germinal cavity against the inferior surface of the blastoderm. A few drops of the extract were mixed with the white above the blastoderm. The fluid could easily be seen to accumulate against the embryo during injection. Abnormal embryos, or those visibly injured during injection, were discarded. Finally, the egg was coated with a sheet of sterile tinfoil and returned to the incubator.

IV. DIAGNOSIS

In order to study the effect of the injected extract, an examination was made on the 4th day after injection. The general shape of the embryo was studied, and an analysis made of the state (normal or abnormal) of the various tissues and organs. The age was determined by means of the sum of these observations. Particular importance was ascribed to the number of somites and the length of the limbs. Most of the embryos had reached stage 22 or 23 at the time of analysis. The heart function was also studied.

In this preliminary examination, the results were classified as follows:

1. The embryo had developed normally. In the tables and figures, such cases are denoted as *N* (normal).

2. Qualitative (or clearly visible quantitative) anomalies of an organ had

appeared, corresponding to the tissue from which the injected product was derived, whereas the other organs and tissues had developed normally. In these cases, it is possible to speak of homologous inhibition; they are therefore denoted as *HI*.

3. Abnormal development had occurred of a different organ or tissue to that from which the extract was derived. This was relatively unusual. In the tables and figures, such cases are denoted as *BI* (embryos whose blood development was partially inhibited, or *NSI* (embryos with a poorly developed nervous system).

4. A small "hump" had formed, which was apt to become filled with blood if there was contact with the adjacent blood islands. This small mass of cells generally pulsated rhythmically. Histologic examination showed that differentiation of, for example, some nervous and chorda tissue had started. These highly defective embryos, which were not differentiated into macroscopically definable organs, are denoted as *U*. Examples of such abnormal cases are shown in Figs. 19 and 20.

Two different factors may be responsible for these abnormalities. The first is mechanical damage; if the extract is injected too rapidly, it may produce distortion of the blastoderm, thereby preventing its normal development. The second factor is chemical; the tissue extracts have a general toxicity, which affects the whole development. The problem is therefore to ascertain whether this general toxicity does, in fact, mask homologous inhibition.

After some experience, it was generally possible to avoid mechanical distortion. As far as general toxicity is concerned, that of brain tissue is not particularly high, and whole extracts diluted in Tyrode solution can be used. The general toxicity of blood extracts is high, but it can be eliminated by fractionation. It was not, however, possible to eliminate the general toxicity of heart extracts, and resort therefore had to be made to diagnostic methods permitting analysis of the "humps" (*U*).

In Fig. 3, showing the results of the early stages of the investigation, these "humps" have been included under a special heading (*U*). In the following figures, dating from the time when more refined techniques were used, they have been omitted, in view of their sparse occurrence.

As stated above, more refined diagnostic methods were applied in the course of the investigation, and the general examination was complemented by more specialized analyses of certain organs. The advantage of the latter is that they are quantitative. This does not imply that the general qualitative or anatomic examination is superfluous, since the two quantitative techniques cannot be used concurrently on the same embryo.

The tests were made on natural populations, and on populations treated with extracts of organs that were non-homologous in relation to the organ being studied (controls), and subsequently on populations treated with extracts of homo-

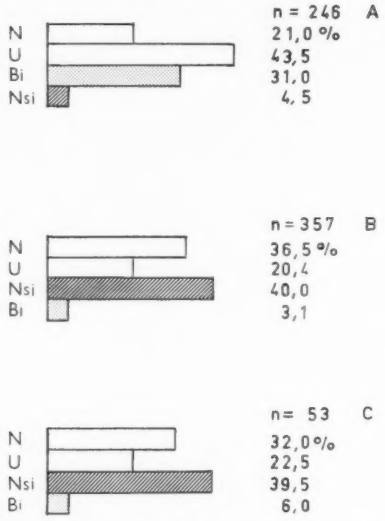

Fig. 3. Action of blood extracts and of brain extracts, evaluated by preliminary (qualitative) method of diagnosis. The length of the staples denotes the percentage of embryos with malformations or with developmental retardation of homologous tissues or organs. A. Blood extracts (cock, chick). B. Brain extracts (cock). C. Brain extracts (chick). *Blood extracts:* marked inhibition of blood development; inappreciable effect on nervous system. *Brain extracts:* marked inhibition of head development; inappreciable effect on blood.—The large proportion of "humps" following injection of blood extracts is due to toxic substance; in later experiments it could be eliminated from the extracts without removing the specific inhibitors. **N** = normally developed embryos. **U** = "humps". **NSI** (shaded staples) = embryos with abnormal cerebral vesicles. **BI** (black staples) = embryos with low Hb concentration.

logous organs. The tests were performed on all embryos (*N, HI, BI, NSI*) except the "humps", which were discarded.

It would perhaps have been more conclusive to present only the results obtained by application of the best methods. However, in view of the importance of having a numerically large material, it has been considered justified to record all the results obtained by use of three groups of diagnostic methods, successively more precise.

Preliminary Qualitative Methods

Blood. At the beginning of the work, only an approximative method was used. The embryos with abnormally pale blood were denoted as — , and those with normally coloured blood for the stage shown by the degree of development of the other tissues as +.

Brain. In the early stages of the investigation, visual examination was used; this is a rapid but imperfect method. When the head was normally developed it was denoted as +, and when it showed anomalies of the cerebral vesicles or

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accessory organs (olfactory, visual or auditory apparatus) as —. The other organs were also classified, and the observations assembled in a table. Despite its imperfections, this qualitative method is of great value, since its results are confirmed by those of the quantitative analysis.

Semi-Quantitative Methods

Blood. This method is a slight improvement on the preliminary method. The precision is increased by the possibility of numerical gradation of the colour of the blood, by comparison with TALLQVIST's colorimetric scale for rapid determination of the hemoglobin content (Hb). In order to avoid errors arising from the fact that the embryo is not always in exactly the same position in the egg, the colour intensity of the blood was measured both in the heart and in the omphalomesenteric vessels, and the average taken. The comparison cannot be made by applying a drop of blood to absorbent paper, since it is too delicate an enterprise to draw blood at the stage in question; moreover, injury to the embryo should be avoided. As a basis for comparison with blood-injected eggs, the variation in Hb concentration as a function of age was studied in normal populations. Populations treated with extracts were then studied, and the results compared with those in the normal (control) populations.

Brain.—Planimetry. Each embryo was placed in a few ml of Tyrode solution, and the right and the left side drawn with the help of a camera lucida. Owing to their transparency, the cerebral vessels and their annexes, the visceral arches, somites and other organs could be drawn. The borderline between head and body was determined as follows. A tangent to the hinder part of the auditory vesicle was drawn from the superior maxilla to the spinal cord, at the level of the first somites. The surface of the projection of the head determined in this way was measured three times with a planimeter. The average values obtained at the different stages of development, both in normal populations and those treated with various extracts, were compared. Although this method is of certain value from the quantitative point of view, it is time-consuming and still far from exact.

Quantitative Methods

Blood.—Benzidine-peroxidase activity. This classical method, used in medical chemistry for detection and measurement of small quantities of blood, is based on the enzymatic activity of blood on H_2O_2 and on benzidine. Provided that the reaction is stopped after a given time, the colour that develops has an intensity which is proportional to the Hb content of the blood sample.

This method was used for determination of the total Hb content of the blood, both in normal embryos and in those treated with organ extracts. The problem of obtaining the blood was solved as follows. The embryo was carefully examined, the condition and function of the various organs were recorded, as well as the age. The egg was then chilled in the ice-box at $\leqq 4°$ C, but was not allowed to

freeze. The blood then became more viscous, and there was no risk of it being lost through rupture of a vessel during removal of the embryo. This was done with a pair of fine scissors, following the contour of the sinus terminalis. The body, with the complete circulatory organs containing all the blood, was then removed from the egg with the help of forceps and a small spatula, and placed directly in a homogenizer. Homogenization was performed with 10 ml of distilled water for 15 minutes. To 4 ml of the homogenate, 1 ml of saturated benzidine and 1 ml of 3 per cent H_2O_2 were added. After 3 minutes, the reaction was stopped by addition of 1 ml of 30 per cent NaOH. The mixture was diluted with 10 ml of 99 per cent alcohol, filtered through Munktell 00 paper, and read off in the photometer.

The curves showing the normal variation in Hb content of the blood in populations at a given stage were plotted. They were used as the basis of comparison in a study of the influence of the extracts on development of the blood in embryos at corresponding stages, both in those treated with blood extracts and those treated with brain extracts (controls). The influence of the action of cytochromes on the results will be discussed later (see p. 181).

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Brain.—Total N. In order to determine the variations in growth of the head in relation to total growth of the embryo during the 3rd and 4th days of incubation, the total N content of the "head" was determined in a large number of embryos, as well as the total N content of the "body". This analysis gives an exact picture of the mass of "head" and "body" tissue, respectively, synthesized at a given stage. The specific action of extracts on the brain can be detected and measured by analysis of the variations in the ratio $\frac{\text{Total N head}}{\text{Total N embryo}}$ as a function of development in normal populations and in populations treated with extracts.

Dissection was performed in a hollow piece of wood containing a small quantity of Tyrode solution. The bottom was coated with paraffin. The membranes of the embryo were removed. If any blood remained in the heart and vessels, it was expelled. Following the same line as that used in planimetry, the "head" was detached from the "body". Each dissected part was placed in a pyrex tube containing 0.3 ml of 10 *M* H_2SO_4 and 4 drops of H_2O_2 (perhydrol) and combusted. Perhydrol was added to secure final combustion. The N content was determined by direct nesslerization. The method gave good results, ranging from about 50 to 200 γ.

Eye

A qualitative diagnosis was made by direct examination and a study of histologic sections. Some precision could, however, be attained by measuring the maximum diameter of the eye in relation to the maximum diameter of the head, and by studying the variations in this ratio as a function of age and treatment. For this purpose, the embryos were placed in a few ml of Tyrode solution, and drawn

in a camera lucida. The maximum diameters of the projected surfaces of the head and eye were measured. These diameters were obtained by drawing a circle in which the surface of projection of the head or eye was inscribed. The variations in the ratio $\frac{\text{Diameter of head}}{\text{Diameter of eye}}$ as a function of development was studied first in populations of normal embryos, and then in populations treated with the extracts. At stages 21—22 (LILLIE's classification), the figure for the normal ratio is around 3.3.

To test the specificity of the influence of the eye extracts, use was also made of an extract of cerebellum, to which an extract of eye (aa) was added, and a control extract of medulla oblongata. The first two extracts had a nitrogen content of 500 γ/ml, that of the medulla oblongata extract being 1600 γ/ml.

Heart

Since the extracts proved to be highly toxic, fractionation was performed by salting out with ammonium sulphate. This method gave good results with blood. Since it was not effective with heart extracts, it was abandoned in preparation of these extracts.

Since most embryos developed very abnormally after injection of heart extract, it was necessary to use a method based chiefly on analysis of the resulting cellular masses ("humps"). A large number of the "humps" were analyzed. To check the specificity of action of heart extract, similar "humps" obtained after injection of brain extract were analyzed. A striking difference could be noted between the "humps" sometimes found in the experiments with brain extract and the cellular masses produced by heart extract. The former were round and, in most cases, pulsated regularly like a heart (Fig. 18). The latter were somewhat elongated, and pulsations were seldom observed.

These pulsations indicated the formation of cardiac muscle fibres. The number of cellular "humps" with such pulsations was therefore counted in a large number of eggs, both those treated with brain extract and with heart extract, and the results compared. These functional observations were complemented in some cases by histologic studies.

Those embryos treated with heart extract which were able to develop were also studied after fixation and staining.

Histologic Examination

The embryos showing typical malformations were drawn when they were still transparent, and then fixed in Bouin's fluid. The thickness of the sections was 8 or 10 μ. They were stained with hematoxylin and eosin. The most characteristic sections were drawn in the camera lucida.

Results

I. BLOOD

Preliminary Qualitative Method

The series of experiments with injection of defibrinated cock blood, washed red corpuscles suspended in Tyrode solution, or hemolyzed blood centrifuged and sterilized by passage through a G5M Jena filter, resulted in many cases in the formation of "humps". In some cases, however, embryos developed which had very pale blood, although other tissues and organs were normally developed.

In order to eliminate the toxic action of whole blood extracts, and to determine the generally toxic agent, as well as the nature of the homologous inhibitor, the donor blood was fractionated.

Series of experiments showed that cock hemoglobin had no specific effect on development of the blood. On the other hand, its general toxicity was great, and most of the eggs into which it was injected failed to develop. This toxicity might have been due to formation of bilirubin and biliverdin. In some experiments, various types of porphyrin were injected, as well as different amino acids (*e.g.* histidine, which has a high concentration in the blood). None of the substances tested had any specific inhibitory effect on blood development.

Both cock and horse serum were tested. They proved to be completely harmless, and the embryos developed normally.

In contrast to the action of these substances, blood extracts from which the Hb had been removed inhibited the formation of blood in a significant number of cases. In order to obtain a large material, all the experiments with blood, as well as with fractions devoid of Hb, have been assembled in Fig. 3, A. In 31 per cent of the cases, blood extracts from the adult had an inhibitory action on development of embryonic blood. Although B and C in Fig. 3 actually derive from another part of the investigation, they are shown here as controls.

The brain extracts used during the same period had no inhibitory influence on development of the blood.

Comparison with Tallqvist's Scale

In order to increase the size of the material, all the results obtained with hemolysates or blood fractions devoid of hemoglobin and nucleoproteins have been combined in Fig. 4. In normal populations at stages 20—22, the maximum Hb concentration amounted to 70—80 on Tallqvist's scale. The populations treated with brain extract showed the same variations in Hb content. This shows that neither operation nor brain extract had any inhibitory effect on blood development.

The embryos treated with extracts of adult blood, on the other hand, showed retarded blood development; the curve had a different shape, with a maximum at

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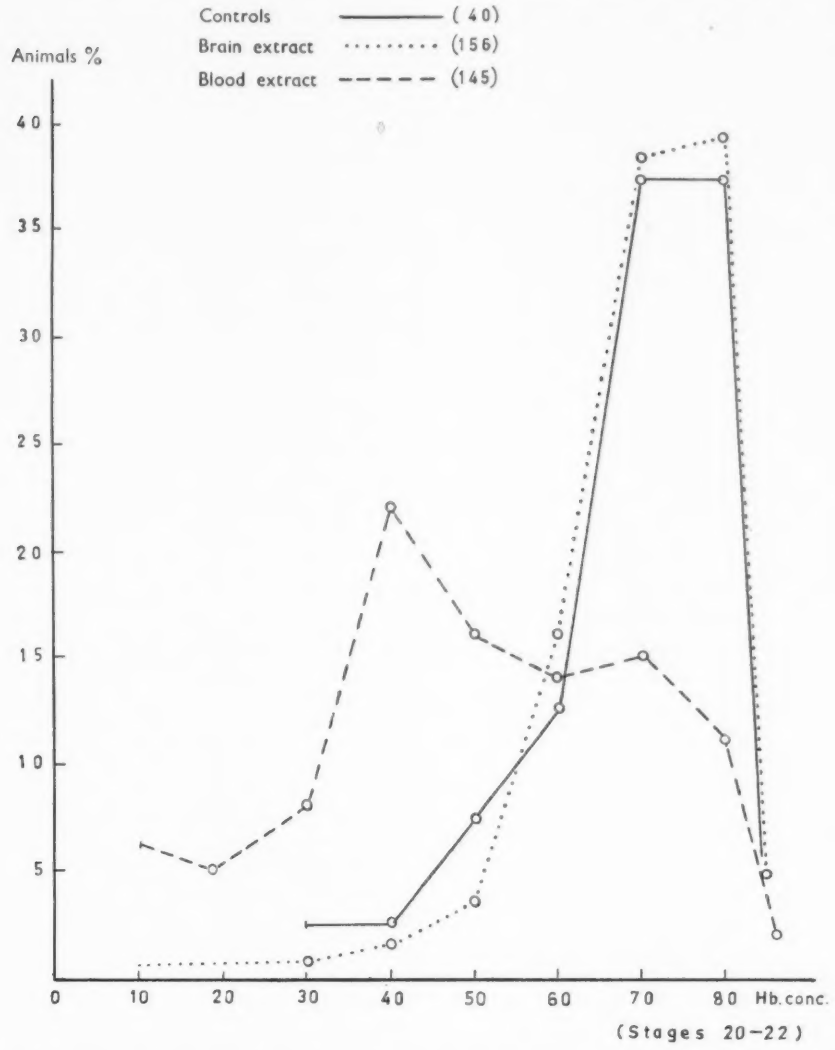

Fig. 4. Action of extracts of adult blood on the formation of embryonic blood: comparison with TALLQVIST's colorimetric scale of Hb concentration. The curve for embryos treated with blood extract is shifted to lower values than curves for controls and for embryos treated with brain extract. Note that the two latter curves completely cover each other.— The "humps" have been discarded.

40 on the scale. The extracts from which the Hb was removed by precipitation with ammonium sulphate also had a specific inhibitory effect on blood development (Fig. 5).

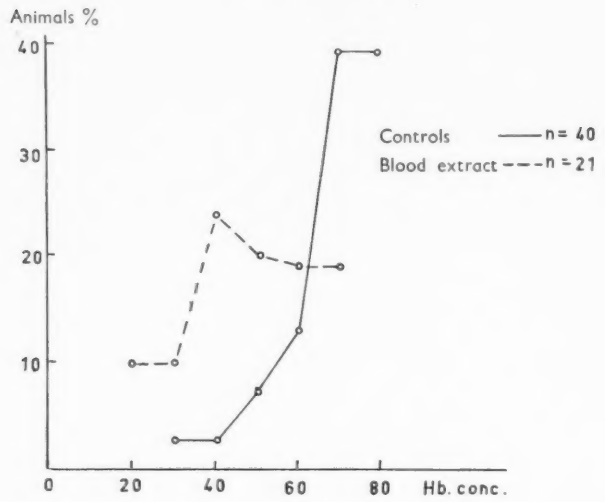

Fig. 5. Action of blood extracts deprived of Hb by precipitation with ammonium sulphate on the formation of embryonic blood: comparison with TALLQVIST's scale. The curve for embryos treated with these blood extracts is shifted to lower values than the curves for the controls.

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Benzidine-Peroxidase Activity

This method was used only in embryos treated with extracts from which the hemoglobin had been removed by salting out. The results were compared with those obtained in normal embryos and in those treated with brain extracts.

Although the number of experiments was not as large as in the previous cases, and the curves were less regular, striking differences were evident. Fig. 6 shows the variations in enzymatic activity at stages 19—20, 21—22 and 23—24 in non-treated populations (middle curve), in populations treated with blood extracts (left-hand curve, lowest values) and in those treated with brain extracts (right-hand curve, highest values). Fig. 7 shows the variation in average enzymatic activity as a function of age in these populations and in some other experiments.

The results obtained by these various determinations seem to be in good agreement, and indicate that the phenomenon of homologous inhibition occurs during early development in the chick embryo.

II. BRAIN

Preliminary Qualitative Method

Altogether 17 series of experiments were made, with a total 357 eggs, using cerebral tissues of varying origin. It was found that the injected eggs almost invariably produced living embryos with regularly pulsating hearts, blood with

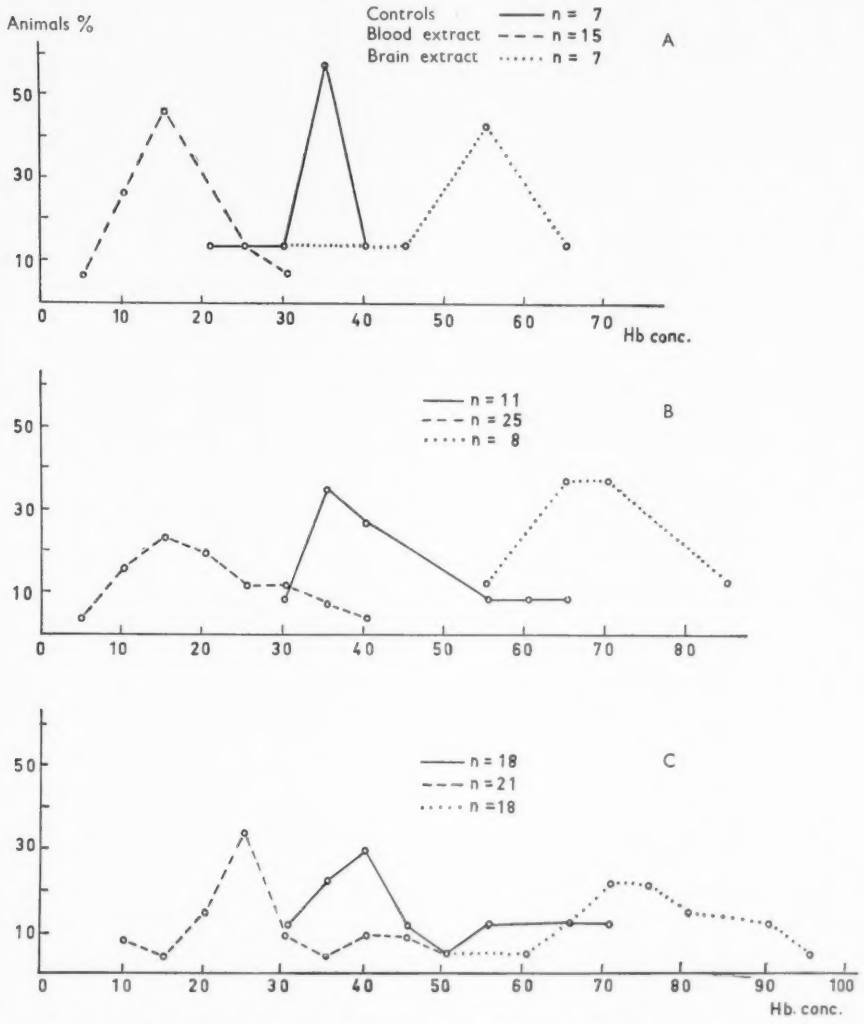

Fig. 6. Benzidine-peroxidase activity of blood (due almost entirely to Hb) of embryos treated with blood extract, of controls and of embryos treated with brain extract. Diagnosis made at three different stages. Blood extract causes inhibition of blood development. Brain extract promotes blood development as compared with controls. A. Diagnosis at stages 19—20. B. Diagnosis at stages 21—22. C. Diagnosis at stages 23—24.

a high Hb content, but with heads that developed abnormally in 40 per cent of the cases (Fig. 3, B and C).

The experiments made with blood extracts can serve as controls; in only 4.5 per cent of the cases they resulted in embryos with specific malformations of the head (Fig. 3).

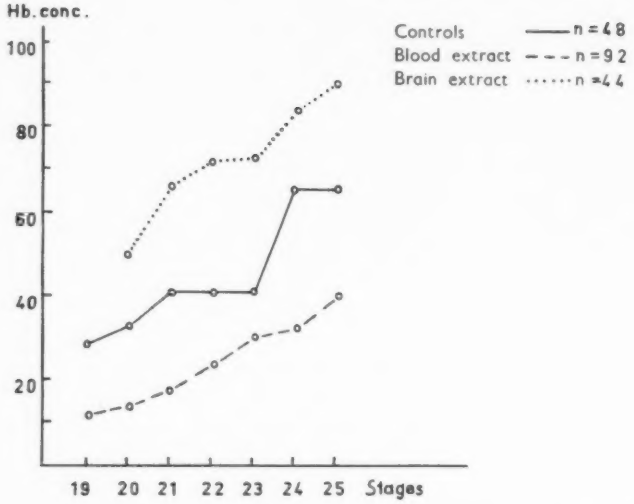

Fig. 7. Curves of the average development of benzidine-peroxidase activity of blood from stage 19 to stage 25: normal embryos (controls), embryos treated with blood extract and with brain extract.

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Planimetry

This method disclosed large individual variations in size of the head at a given stage, not only in populations of treated embryos, but also in normal populations. The method is not particularly accurate, and it would have been very time-consuming to collect the considerable material necessary to establish the exact range of these variations. The average figures found at stages 18—21, both in populations treated with brain extracts and in control populations, are, however, given in Fig. 8 as preliminary data. These figures are based on series comprising 81 eggs into which brain extract was injected, 40 non-treated embryos (controls) and 39 in which either blood or heart extract was injected.

Fig. 9 represents the variations in the projected surface of the head in a population treated with brain extract and in a control population of the same age (stage 21). It can be seen that the curve for the treated embryos is slightly displaced towards the lowest values.

Quantitative Chemical Analysis

The variations in the ratio $\frac{\text{Total N head}}{\text{Total N embryo}}$ gives an objective picture of the variation in growth of the head in relation to the body, as a function of development.

The differences between the average of the individual values in normal embryos and in those treated with brain extract show that this extract had a charac-

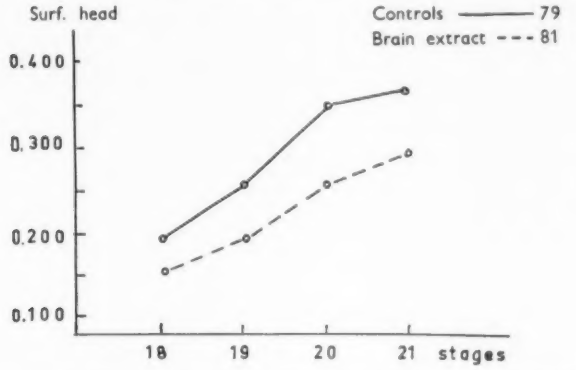

Fig. 8. Action of brain extract on development of the head. Planimetric determination of development from stage 18 to stage 21. The curve for the treated embryos is shifted to lower values.

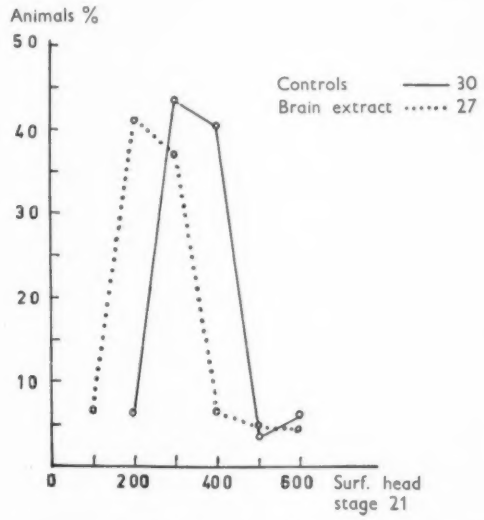

Fig. 9. Action of brain extract on development of the head (planimetry). Population curve for stage 21.

teristic inhibitory action on development of the head. This action was not necessarily associated with morphologic anomalies, as can be inferred from Fig. 10. In this figure, the first colum shows the average normal values, which increase successively from stage 20 to 23. The other columns represent treated embryos. The second column (vertical hatching) denotes those embryos in which morphologic examination disclosed no pathologic features, apart from slight, specific inhibition of the rate of development of the head, which in these cases was the "young-

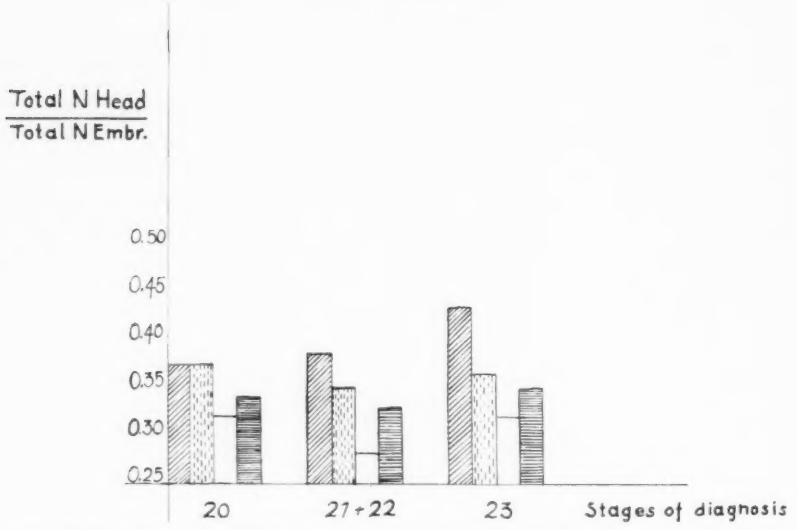

Fig. 10. Action of brain extract on development of the head: variations in mean ratio $\frac{\text{Total N head}}{\text{Total N embryo}}$ as a function of stages of development in control populations (////) and in populations treated with brain extract (═══). The treated populations are divided into those of normal appearance (▒▒▒

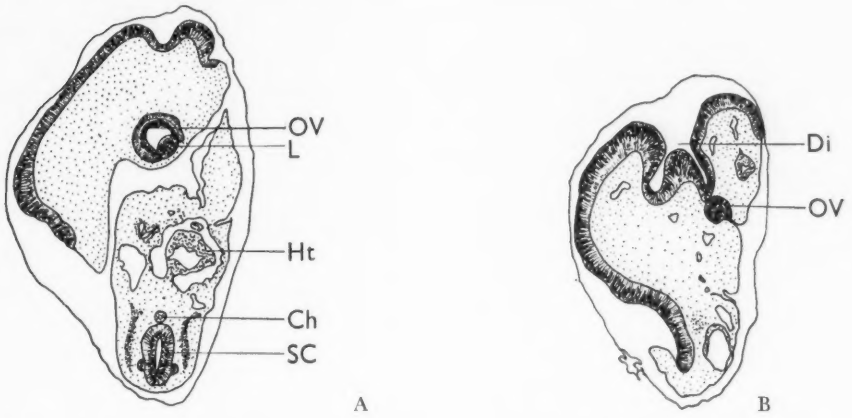

Fig. 11. Action of brain extract on development of the head. Examples of anomalies denoted as type 1. In A and B, the sections pass through the 2nd cerebral vesicle. In A, the section also passes through the body at the level of the heart.—The head is small, the folds of the neural plate have not fused at the level of the 1st and 2nd cerebral vesicles, and the eyes are atrophied. Di: 2nd cerebral vesicle. OV: optic vesicle. L: lens. SC: spinal cord. Ch: chord. Ht: heart.

Anatomic Examination

The malformations produced by injection of brain extract could be divided into the following three main types:

Type 1. The head was abnormal; the folds of the neural plate had failed to fuse completely. Body development was normal, and the heart beat regularly. Examples are shown in Fig. 11, A and B. It is of interest to compare these sections with those from embryos of corresponding age in DUVAL's *Atlas d'Embryologie*.

These sections are from embryos treated with brain extract. The sections pass through regions corresponding to the 1st and 2nd cerebral vesicles. The head has a large opening, and the neural plate is spread around the ectomesoderm. The 1st vesicle has given rise to an optic vesicle, and a lens has formed, but the eye is small (A) or greatly distorted (B). The spinal cord is normal, as are the heart, the somites and the rest of the body (A).

Type 2. In this group, the cerebral vesicles were closed, but remained small and poorly differentiated. The head was small in relation to the normally developed body. Examples of such embryos are shown in Fig. 12, A—D. In A and B, both eye and nose are lacking. In C and D, both eyes and nose are present, but one eye is smaller than normal. The auditory vesicles are well developed.

In Fig. 13, A and B, the sections pass through the head of embryos in which the 1st and 2nd cerebral vesicles have failed to develop. In A, the 3rd vesicle and

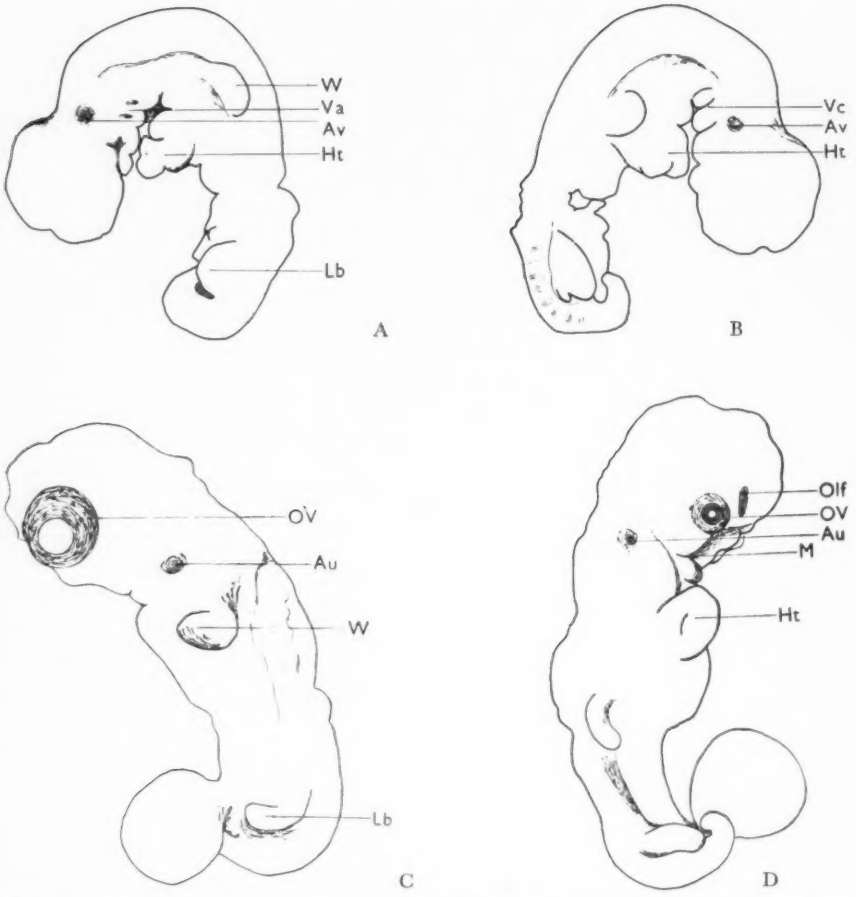

Fig. 12. Action of brain extract on development of the head. Examples of anomalies denoted as type 2. Whole embryos seen from both right and left sides. A and B, embryos at stage 21; C and D, at stage 23.— Head markedly undersized. In A and B, both eye and nose are lacking; in C and D, one eye is undersized. OV: optic vesicle. Olf: Olfactory pit. Au: auditory vesicle. M: mouth. Vc: visceral clefts. Ht: heart. W: wing bud. Lb: leg bud.

auditory vesicles are normal, and the gills and mouth are well developed. In B, the 3rd vesicle is greatly atrophied, but the spinal cord is normal.

Type 3. The aforementioned types of malformation were associated with abnormal fusion of the head and heart, *i.e.,* omphalocephaly. The head was atrophied, the three cerebral vesicles were abnormal or fused, and their annexes (nose, eye, ears) were often lacking (Fig. 14, B and C). The malformation or absence of visceral arches may, however, have been of secondary origin. It should, however, be noted that, even if the shape of the heart was abnormal, it was well developed

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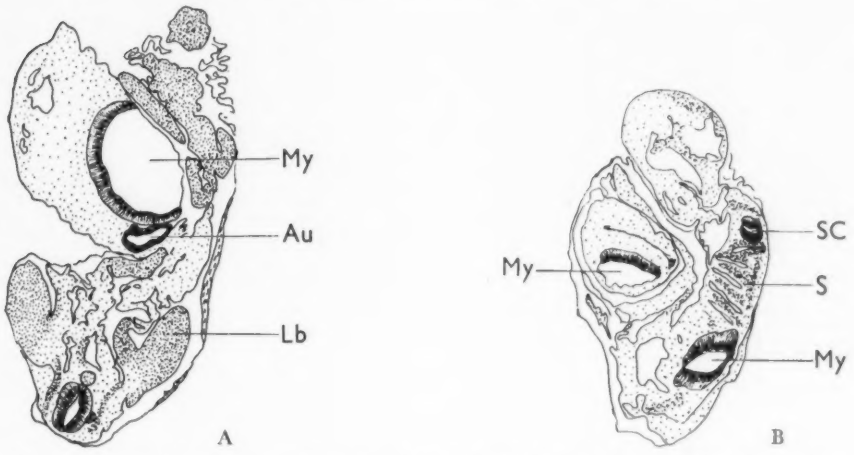

Fig. 13. Action of brain extract. Sections through the 3rd cerebral vesicle of embryos showing anomalies of type 2. My: 3rd cerebral vesicle. Au: auditory vesicle. SC: spinal cord. S: somites. Lb: leg bud.

with respect to the amount of cardiac tissue, and its function was normal (*cf.* p. 188).

Influence of Concentration

Brain extract was prepared in the usual way. The injectable product obtained after filtration through a sterile G5M Jena filter contained 700 γ N/ml; it was found to be active on biological assay. Diluted to 1 : 10 with sterile Tyrode solution, it still proved to be active. Its inhibitory action on brain formation was, however, lost in a dilution of 1 : 25 (1 ml = 140 γ N) : *cf.* Table I.

Table I

Action of different dilutions of brain extract

Dilution (γ N ml)	N %	HI %	U %	n
700	50	37	13	30
350	43	38	19	21
140	69	5	26	22

N: normal embryos
HI: brain inhibition
U: poorly developed embryos
n: number of hosts

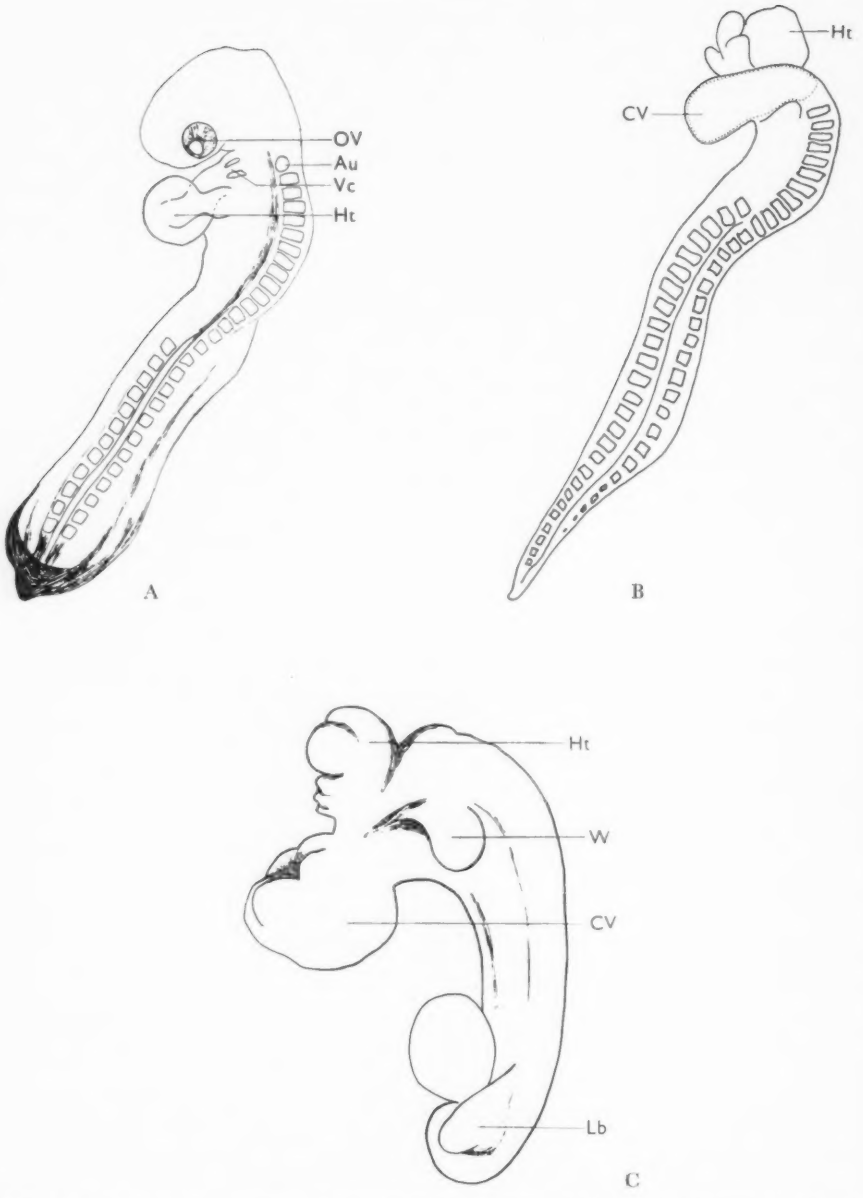

Fig. 14. Action of brain extract. Examples of omphalocephaly (denoted as type 3), which was relatively infrequent. A. Normal embryo at stage 19. B. Treated embryo at same stage. C. Treated embryo at stage 23.— The head is undersized, as well as anatomically abnormal. The heart, although morphologically abnormal, is well developed and functions normally. CV: cerebral vesicle. OV: optic vesicle. Vc: visceral clefts. Ht: heart. W: wing bud. Lb: leg bud.

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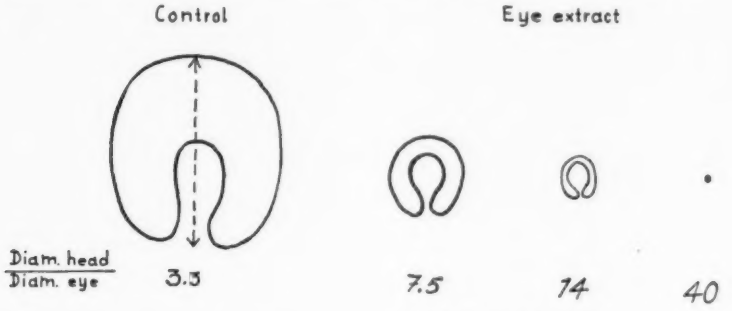

Fig. 15. Action of eye extract. Variations in the ratio $\frac{\text{Diameter of head}}{\text{Diameter of eye}}$ in normal embryos (first diagram) at stages 21—22 and in embryos treated with eye extract (other three diagrams) at same stages. The quotient is about 3.3 in normal embryos, and 7.5, 14 and even 40 in treated embryos.

III. EYE

The action of extracts of substances deriving from the eye was highly characteristic. Although they also affected development of the head, such malformation was invariably associated with partial or total inhibition of formation of the eye. In certain cases, only development of the eye was inhibited, or the eye was poorly developed. Such inhibition or maldevelopment of the eye was present in 50 per cent of the cases.

The inhibitory effect varied not only with the different extracts, but also with one and the same extract, probably depending on the age and receptivity of the host. Some idea of the variation in intensity of action of the extracts could, however, be obtained by comparing the ratio $\frac{\text{Diameter of head}}{\text{Diameter of eye}}$ in embryos at stages 21—22 treated with various extracts, and the normal ratio at these stages, *i.e.*, about 3.3 (*cf.* Fig. 15). Examination of 25 embryos with malformed eyes permitted classification into three groups, with a quotient of 7.5, 14 and 40, respectively. The last figure represents cases in which the eye consisted merely of a pigmented spot. It is, however, important to note that a lens was developed in many cases (Fig. 16, B).

The action of combined brain and eye extracts was intermediate to that of separate extracts of these tissues.

Anomalies in formation of the head were always associated with partially inhibited eye development. In the head, the nervous tissue was either normally developed or, if malformation was present, a large number of folds were sometimes visible (Fig. 16, A).

An embryo with an abnormal head is shown in Fig. 16, A; the neural plate was incompletely fused, and no lens had been formed. The body was normal, but the whole embryo was undersized. In another case (Fig. 16, B) only one eye was

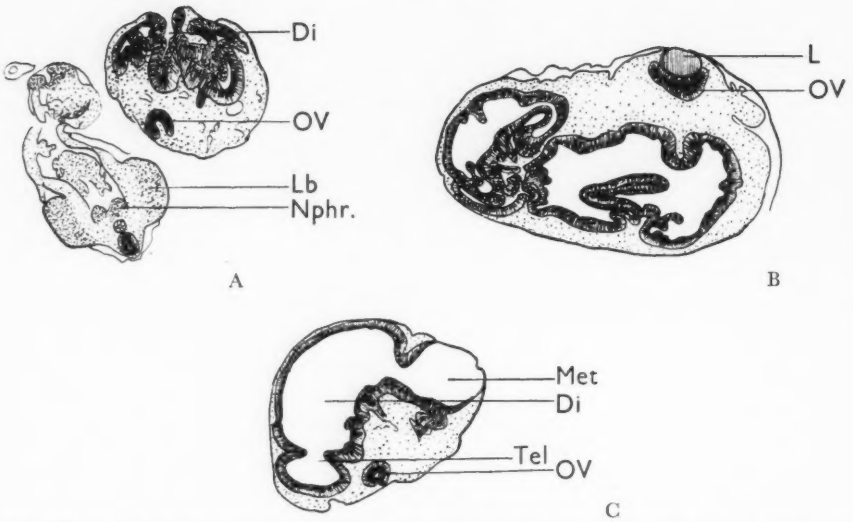

Fig. 16. Action of eye extract. A. Section through the 2nd cerebral vesicle; the section also passes through the body. The neural plate is incompletely fused. The optic vesicle is small and abnormal, and no lens is present. B. Section through the 1st and 2nd cerebral vesicles. The nervous tissue is fairly abundant. There is only one eye; the optic vesicle is small, but the lens normal. C. Longitudinal section through the head. The head is normal except for the eye, which is fairly small and lacks a lens. Tel: 1st cerebral vesicle. Di: 2nd cerebral vesicle. Met: 3rd cerebral vesicle. OV: optic vesicle. Lb: leg bud. Nphr.: nephros.

present and was malformed; the optic vesicle was rudimentary, but the lens was normal. The optic vesicle on the opposite side was not differentiated, and no lens was present. The body was normal. In the embryo shown in Fig. 16, C, the brain was normally developed but the eyes were malformed; the optic vesicle was fairly small and no lens was present.

Controls treated with brain extract having a high content of white matter (extract of medulla oblongata) displayed malformations of an entirely different nature. In some cases the folds of the neural tube did not fuse normally, whereas in other cases development proceeded normally up to stage 21, after which death occurred. Fairly characteristic features in these cases were that blood collected in the region of the gills, and the 3rd ventricle was malformed. Until a larger material has been studied, it cannot be stated with certainty whether or not this extract of white matter caused homologous inhibition of the 3rd cerebral vesicle. It is, however, evident that the extract influenced neither development of the first two cerebral vesicles, nor formation of the eyes.

IV. HEART

1. In those embryos treated with heart extract which reached stages 21—22, differentiation of the heart tube was delayed. Instead of being bent, with di-

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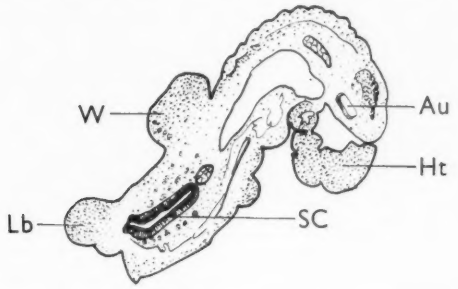

Fig. 17. Action of heart extract. Embryo at stage 22+. Longitudinal section through the heart region. Heart development is retarded, *i.e.*, it is relatively less developed than limbs. Somites small.

stinctly differentiated chambers, it had remained fairly straight, with a fairly constant diameter.

The blood was often pale, and the somites undersized. There was, however, frequently abnormal development of the head as well. In order to demonstrate a specifically anti-mesodermal effect, these results must be compared with those obtained in experiments with brain extracts. The latter produced anomalies in development of the head alone, whereas the blood, somites and heart were generally normal. In the few cases in which there was cardiac malformation on mechanical grounds (omphalocephaly), it was of neither a histologic nor a physiologic nature; the quantity of cardiac tissue, as indicated by the heart volume, was normal, as was heart function (Fig. 14).

Sections taken at stage 22+ in an embryo treated with heart extract are shown in Fig. 17. (It was not possible to include the heart and head in the same section.) It should be noted that the head is deformed. One of the auditory vesicles, the neural tube and the spinal cord can be identified. The somites are small, but the limbs are normal. Development of the heart, which pulsated normally, was retarded; it had remained at stage 20.

2. In most cases, injection of heart extract resulted in formation of "humps" which, on section, proved to contain large vesicles (*U*). As can be inferred from Table II, the percentage of such vesicles in which pulsations were present was considerably lower than in the "humps" produced by injection of brain extract.

Some of the "humps" were studied histologically. Examples of such sections

Table II

Number of vesicles showing pulsations on the two following media

Heart extract	Brain extract
n = 57	n = 176
13 %	41 %

n: number of hosts

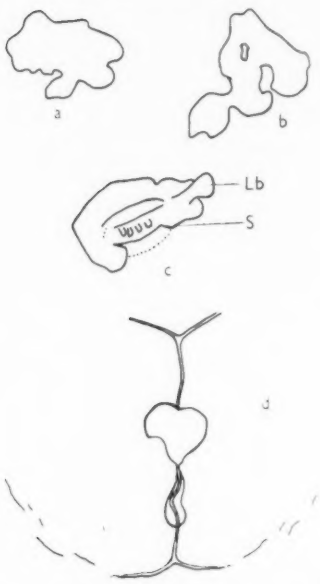

Fig. 18. Action of brain extract. Examples of "humps" occasionally formed. a and b. Pulsative vesicles. c. Somites and extremities recognizable. The whole zone indicated by ..... showed pulsative movements. d. Characteristic pulsative cardiac vesicles accompanied by a circulatory system. Abundant blood is present in all these formations.

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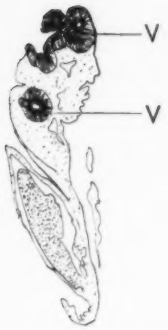
are shown in Figs 19 and 20. Fig. 19 was taken at random from the non-pulsating vesicles produced by injection of heart extract. Fig. 20 represents a random sample of the pulsating vesicles resulting from treatment with brain extract. In Fig. 19, the mesenchyma, a closed neural tube and a large cerebral vesicle open at one point are visible; neither heart nor any blood is present. In Fig. 20, some fairly loose mesenchymal tissue surrounding a cavity in which some red cells are still present can be seen; in the living embryo it was filled with blood. Some large endodermal cells are also visible. The nervous tissue is represented by a short medullary canal. In other sections, the canal is accompanied by a cord, and it then has the characteristic elliptic shape (*cf.* HOLTFRETER's experiments on the morphogenesis of the neural tube in the presence or absence of the cord and the somites). (15, 17.)

The muscles of the pulsative vesicles were not yet histologically differentiated. This is in conformity with the results of PATTEN, LEWIS and others, showing that physiologic differentiation precedes histologic and morphologic differentiation. It is, however, known that some fibrils are discernible in the electron microscope (see review by COPENHAVER, in *Analysis of Development*). (5.)

It can be inferred from the percentage of pulsative vesicles given in Table II and from the histologic examination that these vesicles (Figs. 18—19) had, how-



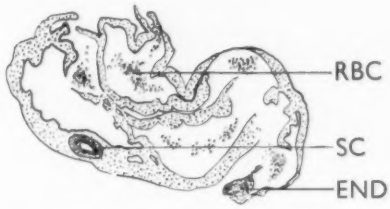


Fig. 19 (left). Action of heart extract. Non-pulsating vesicle. There is no blood, but brain nervous tissue is present (V).

Fig. 20 (right). Action of brain extract. Pulsative vesicle. Abundance of mesodermal tissues and blood; nervous tissue greatly reduced. SC: spinal cord. END: endoderm. RBC: red blood cells.

ever, already undergone some differentiation. Moreover, the nervous tissue developed well in the "cardiac" environment, whereas the cardiac tissue—at any rate the contractile fibrils detectable in the electron microscope— developed well in a "neural" environment, but differentiated poorly in a "cardiac" environment.

V. INFLUENCE OF AGE OF DONOR

An account has already been given of investigations of the influence of the age of the host in studies of homologous inhibition by injection of tissue extracts containing inhibitory substances (see pp. 145—146). The host proved to be receptive at stages 5—6 LILLIE's classification (Fig. 1). Prior to stage 5, it is too fragile to withstand the intervention, whereas after stage 7 it is resistant to the action of the extracts.

It was also considered desirable to determine the influence of the age of the donor, *i.e.*, the time at which the inhibitory substances appear in the tissues. Extracts were therefore made from tissues of donors of varying age, namely, adults, 8-day-old chicks, and embryos aged 12, 6, 5 and 4 days, respectively. The experiments were confined to extracts of brain tissue. The preliminary (qualitative) method of diagnosis was applied, the embryos treated being classified according to their general condition.

Donors 8 Days Old or Above (After Hatching)

It could be expected that the amount of substance inhibiting or promoting growth, respectively, would vary with the age of the donor. Consequently, brain tissue from a 3-month-old cock could be anticipated to have a greater inhibitory effect on formation of the homologous organ of an embryo than that of a chick a few days old. The results of experiments with brain extracts from donors rang-

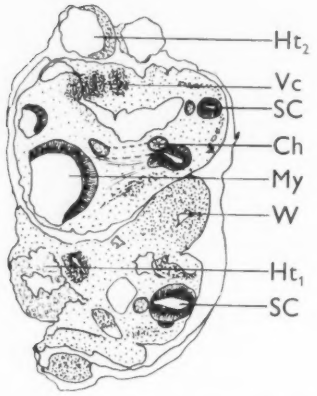


Fig. 21. Action of brain extract. Embryo at stage 23 (*cf.* p. 188). The heart Ht_1 is hypertrophied, and an extra heart (Ht_2) is present.

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ing in age from 8 days to 3 years showed, however, that this is not the case in reality. They had, in fact, the same action.

Donors 4—12 Days Old (After Beginning of Incubation)

The action of brain extracts from embryos aged 4, 5, 6 and 12 days was studied. In the first series, extracts were made from the brain of 15 donors aged 4 days. Altogether 27 donors were used in the second series, and 6 in each of the two last series. The extracts used contained the same quantity of N per ml as the extracts of adult tissues, except for that prepared from 4-day embryos. In this case, the desired concentration could not be obtained; the N content was only 176 γ/ml, and the inhibitory action is known to disappear at around this concentration (*cf.* Table I). The results of all the series are summarized in Table III.

The homologous inhibitory action of brain extracts from donors younger than

Table III

Action of brain extract from donors of increasing age

Age in days of incubation	N %	HI %	U %	n
4	76	12	12	17
5	68	12	20	90
6	52	28	20	25
12	40	44	16	79
>12	37	40	20	357

N: normal embryos
HI: brain inhibition
U: poorly developed embryos
n: number of embryos

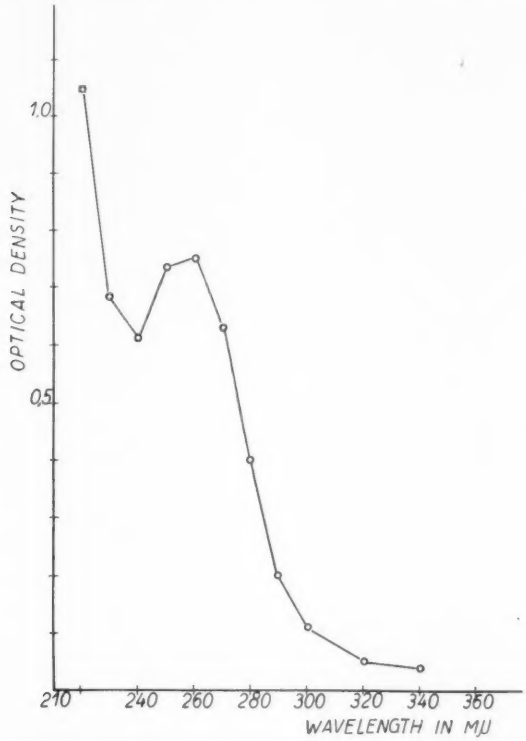

Fig. 22. Ultraviolet absorption spectrum of filtrate of aqueous brain extract from 5-day-old embryos, diluted to 1 : 10 at pH 7.2.

6 days after the beginning of incubation was inferior to that of corresponding extracts from 12-day embryos.

Extracts of mesodermal tissues from 5-, 6- and 12-day-old embryos used as controls had no specific inhibitory influence on development of the head, muscles or blood of the host.

A special property of the brain extract from 5-day embryos must be pointed out, namely, that it accelerated general development of the embryos. Thus, some of them had reached stage 23 or 24 ($> N$ in Table IV) at the time of diagnosis, instead of being at stage 21 or 22. This property may have been due to the high nucleoprotein content of this extract. Fig. 22 shows the UV absorption spectrum of the extract diluted to 1 : 10 at pH 7.2. The peak is at 260 mμ, and the curve is, in fact, typical of the nucleoproteins (*cf.* p. 176, action of fractions with a high nucleoprotein content).

To sum up, it can be stated that the inhibitory activity is present already on the 5th day of incubation, although it is then very weak. It increases distinctly

between the 6th and 12th day, but subsequently remains constant throughout life.

VI. NATURE OF THE HOMOLOGOUS INHIBITORS

The existence of homologous inhibition has been illustrated by the experiments described in preceding sections. The question then arises of the chemical nature of these inhibitors. Only the results of preliminary studies can be presented here. This is because it would require several years to carry out experiments on sufficiently large material, using fractions of extracts that are satisfactorily pure and chemically analyzed.

Dialysis

With a view to eliminating the toxic products which greatly disturbed the experiments made with blood, fractionation of the blood with ammonium sulphate was frequently performed. The filtrate was treated by prolonged dialysis. The core remaining after dialysis invariably had inhibitory properties.

Some experiments were made with extracts of brain tissue or blood that had been dialyzed against distilled water. The core had an inhibitory action, whereas the dialysate had no such action. This indicates that homologous inhibitory substances are non-dialyzable (*cf.* Table V, Fig. 5). Similar dialysis experiments were made with unfractionated extract; the results in such a series are shown in Table V.

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Heating

Brain extracts were prepared in the usual way, and the homogenates heated on the water bath for 15 minutes. The following temperatures were used successively with different fresh extracts: 100° C, 80°, 75°, 60°, 55° and 45° C.

Table IV

Action of brain extract from donors aged 5 days
(after the beginning of incubation)

Concentration in N	N	> N	HI	U
540 γ/ml	8	6	1	4
270 γ/ml	7	1	1	2

Table V

Action of the core after dialysis of brain extract

N	HI	U
9	7	8

Table VI

Effect of heat on the inhibitory action of brain extract

°C	N	HI	U	n
⩽55	80	5	15	118
45—37	47	31	22	64
4	39	40	21	30

Table VII

Action of protein fraction of brain extract (petroleum ether 50°—70° C)

N	HI	U
14	1	6

Table VIII

Action of fractions of brain extract (ethyl ether < 45° C)

	N %	HI %	U %	n
Proteins	52	26	22	23
Lipids	55	4	41	27

The extract was found to lose its activity at about 50° C. The results of the different series of experiments are recorded in Table VI. It can be inferred from this table that the percentage of abnormalities in the embryos treated with extracts heated above 50° C remained within the limits of the natural percentage of specific abnormalities, which is about 5 per cent for both brain and blood.

Treatment with Organic Solvents

In the first experiment, brain fractions were extracted in a Soxhlet apparatus for 30 minutes with petroleum ether (dist. at 50—70° C). The protein fraction remaining after extracting the lipids was homogenized in Tyrode solution, and injected into 21 embryos. It had no specific action on head formation (Table VII). This only confirmed the results of the experiments made with heated extracts.

Another experiment was made with ethyl ether (dist. below 45° C). Extraction was continued for 30 minutes in the Soxhlet apparatus, and the remaining protein fraction homogenized with Tyrode solution. The homogenate was centrifuged and sterile-filtered. The solvent used for extraction of the lipids was evaporated at low temperature, and the brownish oil remaining in the bottom of the flask was mixed with Tyrode solution. It was, however, very difficult to mix, and was certainly incompletely dissolved. The embryos were divided into two groups, for injection with proteins and lipids, respectively. The results showed that the active substance remained in the residue after extraction of the lipids (Table VIII).

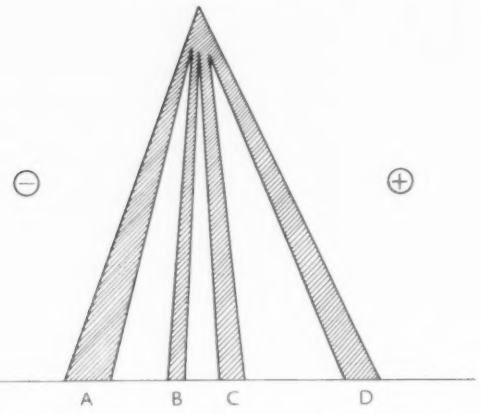

Fig. 23. Electrophoresis of brain extract in phosphate buffer at pH 8.2. Four main fractions were obtained.

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*Separation by Electrophoresis**

The extracts were prepared by homogenization of chick brain tissue in a phosphate buffer at pH 8.2. The salt concentration was 0.03 Mol. Five ml of extract were used, and electrophoresis was performed at 200 volts for 24 hours. Four fractions were obtained: A, B, C and D (Fig. 23). Fractions A and D were studied.

Fraction A had a high lipoprotein content. It was highly fluorescent in UV light; its absorption spectrum (in aqueous solution at pH 7.2) measured in UV light at photometry did not show a characteristic curve. In the electric field, the fraction behaved as if it had a slight positive charge. Fraction D was rich in proteins. It was not fluorescent, and the UV absorption spectrum indicated the presence of nucleoproteins in the mixture obtained (Fig. 24).

The biological activity of fractions A and D was studied. The phosphate was eliminated by dialysis against distilled water, and the core was lyophilized. The powder obtained was dissolved in Tyrode solution, and the resulting solution sterilized by filtration. Experiments with varying concentrations of the solution (measured in γ N/ml) allowed determination of the activity of the extracts.

Fraction A, with a high lipoprotein content, showed no inhibitory action on brain formation. Fraction D—rich in proteins—had, on the contrary, a marked inhibitory effect. Experiments with rising dilutions showed that the inhibitory action of fraction D was at least 10 times greater than that of the original brain extract. Fraction D was, in fact, active in dilutions at which the whole extract was no longer active (140 γ N/ml). The activity of fraction D was still distinct at an N concentration as low as 70 γ/ml. (Table IX.)

The experiments were complemented by quantitative analysis of the ratio

* Apparatus for continuous electroforesis (Elphor. Bender and Hobein).

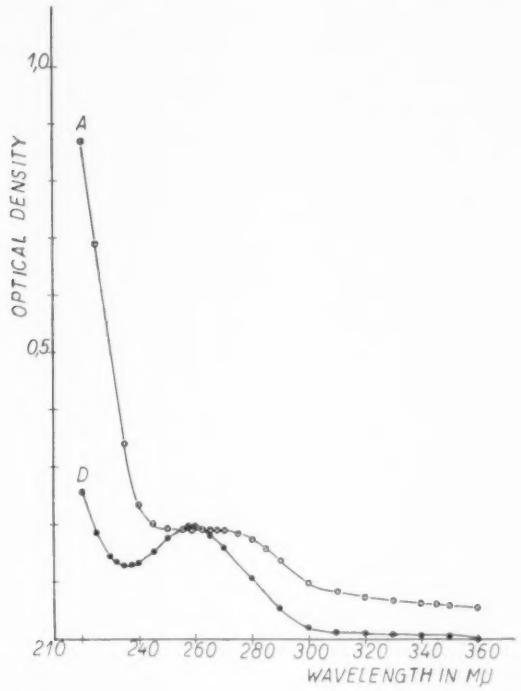

Fig. 24. Ultraviolet absorption spectra of fractions A and D, obtained by electrophoresis of whole brain extract. The inhibitory activity is localized to fraction D.

Table IX

Action of fractions of brain extract obtained by electrophoresis

	N %	HI %	U %	n
Proteins	46	37	17	59
Lipoproteins	44	9	47	79

$\frac{\text{Total N head}}{\text{Total N embryo}}$. Twenty treated embryos were dissected, and determination of the total N content of the head and of the body deprived of the head, respectively, was performed by combustion and direct nesslerization. The mean quotient for the treated embryos which had reached stages 21 and 22 was 0.335, as compared to 0.38 in the controls.

The results confirm those of other types of experiment presented in this section of the present paper. Thus, the homologous inhibitory activity is, in fact, localized to a certain fraction of the organ extract.

Different Protein Fractions

The results of the experiments reported above, particularly those with extracts heated to different temperatures and with separation by electrophoresis, seem to indicate that the inhibitory substances belong to the protein group. Purification of the presumed inhibitors of a protein nature was therefore started by eliminating the nucleoproteins, which are known to have a promoting influence on the growth of fibroblasts in tissue cultures (KUTSKY 1953). (19.)

The donors were 12-day-old embryos. The embryos were removed aseptically from their membranes, and the brain dissected out. Extracts of brain and body were made with the metod described by KUTSKY (19), except that the nucleoprotein fraction (NPF) was precipitated once only with streptomycin in a borate buffer at pH 7.1. The precipitate was centrifuged at 24,000 *g* for 10 minutes, homogenized with a phosphate buffer, pH 7.2, and dialyzed against phosphate and then against distilled water. The white powder obtained by lyophilizing the core was dissolved in Tyrode solution to a concentration of 2 mg/ml, and injected.

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Nucleoprotein Fraction (NPF)

Results with qualitative (+and —) method of diagnosis.—The NPF of brain was tested in 86 eggs. Anatomically, they developed regularly, but more rapidly than normal eggs. Contrary to the expectations, the NPF did not act specifically on head development, but had a generally promoting effect on growth of all organs. In several cases, the head of the embryo was malformed. The NPF also accelerated differentiation even when injected into late neurulae at stage 8 and over. The size of the embryos corresponded to the stage of development.

The 86 blastoderms developed into 23 vesicles ("humps" = *U*), 45 normal embryos, and 18 embryos with abnormalities of the head. Of the embryos able to develop, 27 were remarkably older in an anatomic sense (> *N*) than the controls.

As a control of the experiments with the NPF of brain tissue, 26 embryos were treated with the NPF of body extracts. Its growth-promoting effect was weaker than that of the NPF of brains from the same donors. It had no toxic action on the head.

The NPF of most mesodermal extracts was—like the extracts of mesodermal tissues tested—more toxic than the NPF of brain. It was less active on the rate of development as well. It can be inferred from Table X that the NPF of brain had a certain inhibitory action on development of the head, whereas it markedly accelerated the general development of the embryos. These actions were sometimes simultaneous. The NPF of the body had, on the contrary, no specific effect on either head or body development. Its growth-accelerating action was weak.

Results with planimetric method of diagnosis.—The NPF of brain was injected into 92 embryos, and the NPF of whole 12-day embryos into 123. Both extracts

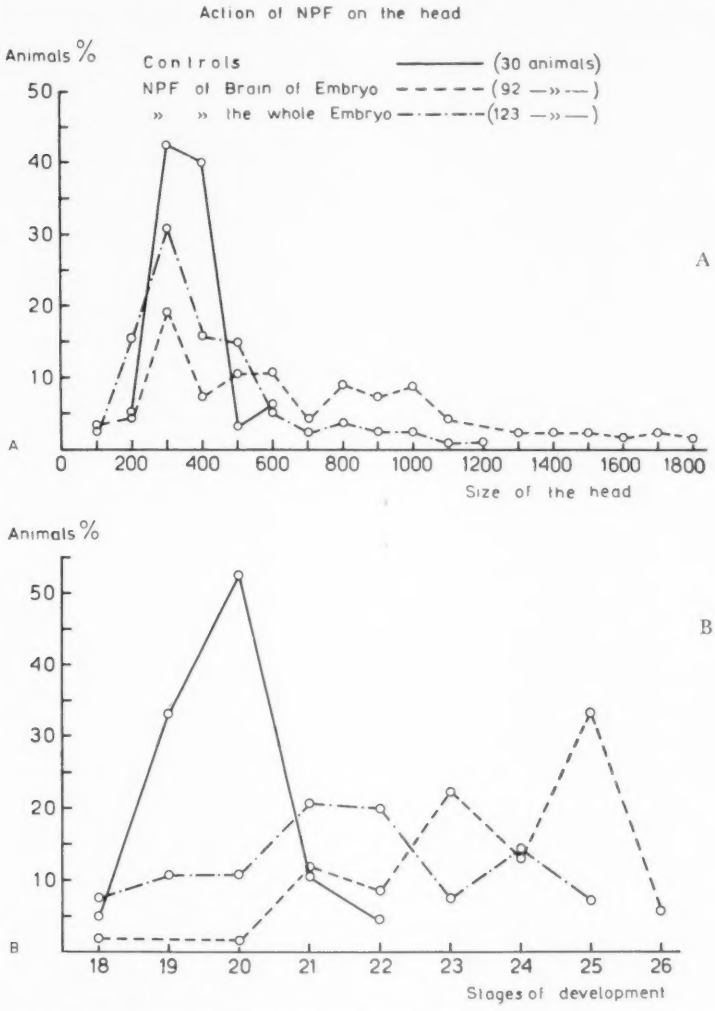

Fig. 25. Action of NPF of brain and of whole embryos on development of the head. A. Planimetry of the projected surface. B. Comparison with LILLIE's table.

had the same effect, but that of the whole embryo was weaker than that of the brain. The shape of the curve for the population of embryos treated with the NPF of whole 12-day embryos (Fig. 25) differs distinctly from that for the embryos treated with the NPF of brain. The size of the head was often twice or more that of normal embryos. Not only were the heads larger than normal, but the whole embryos were more developed than the controls. The NPF thus acted on the rate both of development and differentiation. It did not act as a template in the

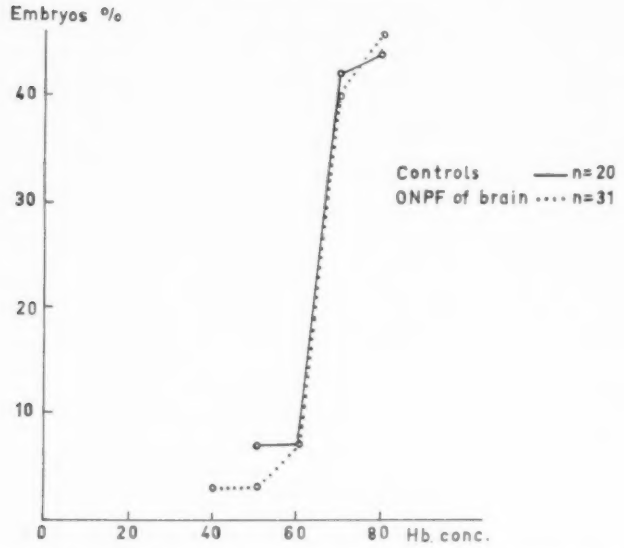

Fig. 26. Action of ONPF of brain on development of blood. This fraction had no inhibitory effect on blood development.

Table X

Action of extracts with high nucleoprotein content (NPF)

	N %	> N %	HI %	U %	n
Brain	21	31	21	27	86
"Body"	51	15	0	34	26

sense of WEISS, *i.e.*, as an organ-specific promoting factor of development. Fig. 25, A shows the curves of variation in size, and Fig. 25, B the variation in anatomic age. These two curves clearly illustrate the way in which the NPF acts on the organism. This effect is similar to that obtained with extracts of the head of 5-day embryos (*cf.* p. 171).

Residue After Precipitation by Streptomycin

Unfortunately, time did not permit numerous concurrent experiments with the residue (denoted as ONPF) obtained after precipitation of the nucleoprotein fraction by streptomycin.

The ONPF of brain was found to have an inhibitory action on development of the head (Table XI). It had no accelerating effect on the rate of general development, nor did it have any inhibitory action on blood development (Fig. 26).

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Table XI

Action of extracts with low nucleoprotein content (ONPF)

	N %	> N %	HI %	U %	n
Brain	60	0	40	0	15
Blood	54	0	46	0	30

Table XII

Action of salt-outed brain extract

N %	HI %	U %	n
48	41	11	36

The ONPF of blood had an inhibitory action on development of the blood (Table XI).

Thus, the nucleoprotein-containing fraction of extracts had mainly a promoting influence on the general growth of the embryos. However, when brain extracts were injected, malformations appeared in 21 per cent of the cases. The fraction presumably devoid of nucleoproteins had a homologous inhibitory effect only.

Brain Extracts After Removal of Most High-Molecular Proteins by Ammonium Sulphate

The ammonium sulphate was added gradually to the extract until saturation occurred. The precipitate obtained was removed by filtration. The whole procedure was performed at 0 to 4° C. The filtered solution was dialyzed to eliminate the sulphate. The core was lyophilized, and the final product dissolved in Tyrode solution.

The solution injected contained 0.5 mg of brain substance per 10 ml of Tyrode solution. It had very little toxic action, no action on the rate of development, and a fairly strong specific inhibitory effect on brain development. This fraction of the brain extract acted on homologous tissue formation in the same way as the corresponding fraction of blood on blood formation (Table XII, Fig. 5).

Discussion

GENERAL ASPECTS

Causes of Variation

The results obtained in large series of experiments were highly characteristic, whereas those in small series were variable. This fact, which made the experiments very time-consuming, may have been due to the following factors:

1. Despite all precautions, mechanical trauma may occur during injection. This cause of variation may be of fairly great importance for abnormal development of the head, since its normal development is dependent on a perfect pattern of morphogenetic movements, and the integration of this pattern is easily disturbed by mechanical distortion at early stages (*cf.* p. 145). For the same reasons, mechanical trauma will, on the contrary, play an insignificant role in development of the blood, as well as of the contractile tissues of the heart, since these tissues develop easily.

2. It seems necessary to establish close contact between the blastoderm and the injected product. If this contact is incomplete, it may produce a variation in the results. Another possible cause is the variation in permeability of the membranes to the inhibitory substances.

3. The individual variability of the embryos is of importance at the moment of injection. It is, in fact, difficult to find blastoderms of corresponding age in the same population. This is important, in view of the fact that the action of the extracts varies considerably with the stage of development of the treated egg. Injection must be made at stages 5—6 in LILLIE's classification. Once stage 7 is past, the extracts no longer have an inhibitory effect on development (see Fig. 1). ROSE (1952) observed an analogous phenomenon when cultivating eggs of *Rana pipiens* in an environment containing cerebral substance of frogs. When treatment took place during gastrulation or at the beginning of neurulation, anomalies typically limited to the nervous tissues occurred. After the beginning of neurulation, the extracts had no effect.

It can also be recalled that the youngest eggs in a certain population, *i.e.*, those whose development is delayed, are the most fragile ones. They are also more fragile than the eggs of the same stage in another population, which is younger.

4. The normal individual variability in development is high during the first week; this is important at the time of diagnosis. The developmental stage of the embryos in a single population is not invariably the same at a given time. The developmental stage of different organs in the same organism is also subject to individual variations. Consequently, it is necessary to study first the normal variations in the organs which are to be the object of experiment, in order to obtain a basis of comparison, and to have previous knowledge of their behaviour.

5. Genetic factors may play a considerable role in variations in the relative size of individual organs. Unfortunately, genetically homologous material was not available in the present experiments.

Masking of Homologous Inhibition by General Toxicity

Dwarfs were sometimes observed among the treated embryos. This seems to indicate the existence in the extracts of a general inhibitor of growth. Such inhibition is apt to mask the action of homologous inhibition. This action was

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unquestionably strongest in extracts of heart and blood, which sometimes caused formation of chaotic organ complexes, denoted here as *U*.

EBERT (1952) observed the same phenomenon when studying the action of antigens of different organs of adult chickens on the development of blastoderms. Thus, he found the antisera to have a general inhibiting action which often masked the homologous inhibiting action. He stated that the specific antibodies retained their activity in dilutions where the general antibodies did not retain their activity. The unmasking of the former could be done by successive dilution. (7)

Fractionation of the extracts was used in the present investigation to try to unmask the specific inhibitory toxicity. In experiments in which the toxicity was decreased by this means, the dwarfish embryos and chaotic formations ("humps") were not numerous; they were therefore systematically omitted from the tables and figures.

I. BLOOD

As early as 1920, SABIN observed that hematogenesis is normally irregular in the chick, and that the surface where the blood islands can appear is large. It is therefore difficult to know whether real and sufficiently long contact has been established, and whether all the hematoblasts have been touched by the injected extract. In similarity to ROSE (1952), it was observed in the present investigation that hematogenesis possessed a great faculty of regulation. ROSE showed, for instance, that the larvae of *Rana pipiens* lose their capacity of forming blood when they are immersed in a dilute solution of blood, but that they regain this faculty when they are transferred to water. In this case, however, the blood islands that have formed in the peritoneum remain isolated, and the blood does not circulate (31).

A similar case of regulation was fairly often observed in the present experiments. The blood islands appeared first in those regions where the injected fluid had been unable to enter. When it spread to the posterior region of the germinative disc—the normal site of blood formation—islands frequently appeared in the region anterior to the area vasculosa.

The question arises of the reliability of the methods used for estimation of the blood content. Both the preliminary qualitative method and TALLQVIST'S semiquantitative method are relatively subjective. Moreover, they measure only the percentage of blood in Hb.

Some objections can perhaps be raised to measurement of the quantity of blood formed by the embryo on the basis of its benzidine-peroxidase activity. This activity is not specific to hemoglobin; it is, for instance, a property of the cytochromes as well. In the present experiments, the benzidine-peroxidase activity was measured in embryos whose blood had been withdrawn prior to homogenization; the activity is very weak in comparison to that in an embryo in which all

the blood is retained. The activity increases successively with growth, but in the early stages, development of the blood is proportionately much more rapid than that of the rest of the body, measured by the benzidine-peroxidase activity of the blood-free body. Thus, if at stages 19—20 the enzymatic activity decreases by 4/5 when the blood has been withdrawn, at stages 22—24 it decreases by 9/10.

It must be pointed out that neither the preliminary, subjective estimation of the blood colour nor comparison with TALLQVIST's scale gave exactly the same results as the method based on the benzidine-peroxidase activity of the blood. In some cases, the two former methods indicated no marked abnormality in Hb concentration, whereas the results with the latter method were clearly pathologic, *i.e.*, definite inhibition had occurred.

A possible cause of this discrepancy is that the qualitative methods give the concentration of the blood in Hb, whereas quantitative determination of the enzymatic activity gives the total amount of Hb. This implies that the blood extracts inhibit development both of the red corpuscles and of the blood vessels which limit the blood volume.

The action on the blood vessels may have taken place in two ways. (1) *Secondarily*. The volume of the heart and vessels, as well as their shape and volume, are known to depend on the quantity of fluid passing through them and on the rate at which it flows (Thomas' law). (2) *Primarily*. The cytological work of SABIN (39) has shown that the first blood cells and first blood-vessel cells have a common origin. The blood extracts may have acted on the common precursor of these cells.

The difference between the results obtained with the respective diagnostic methods was still more marked in the studies of blood development in populations treated with brain extracts. The methods based on the blood colour hardly allowed demonstration of a promoting action on formation of blood, whereas the benzidine-peroxidase method showed conspicuous stimulation (see Figs. 6 and 7). The results obtained with these different methods of diagnosis were not contradictory. On the contrary, they complemented each other, and tended to increase the precision.

Both the material studied after blood injection and the corresponding control material were large, even if only the results obtained with a single method are considered. This material indicates the existence of a mechanism of inhibition of blood development in embryos treated with extracts of adult blood. When unfractionated extracts are used, this inhibition may be masked by the general toxicity of the blood.

II. BRAIN

Experiments with brain extracts must be regarded against the background of the fact that even injection of "neutral" substances, *e.g.* Tyrode solution and

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horse serum, may produce abnormalities—in contrast to conditions in blood and heart tissues—for the reasons discussed above (*cf.* p. 180).

The methods of diagnosis used can be discussed first. The qualitative (visual) method takes into account the qualitative development of the head alone, and provides no information regarding the actual quantity of proteins produced in this part of the organism. It does, however, give a preliminary indication, and the results were confirmed by more exact analyses. The figures obtained were expressive, *i.e.,* 40 per cent of malformations of the head, as compared to 5 per cent of malformations of other organs.

In the quantitative method of analysis subsequently used, the dividing line between head and body is an arbitrary one. Thus, part of the medulla oblongata, containing part of the central nervous system, the spinal cord, is included with the body. However, when it is necessary to study a large number of embryos, in order to obtain characteristic results, this method has great advantages. This is because the auditory vesicle—an important landmark when drawing the dividing line—is always visible in normal embryos up to stage 24, and the joint of the future jaw is also easily identified. It is true that errors may occur when this line is drawn, but it seems likely that they will compensate for each other when a large number of embryos are used. In the rare cases in which the auditory vesicle failed to develop, or was insufficiently developed, the first somites were used as the landmark for drawing the dividing line. It can be pointed out that retarded development of the auditory vesicle is, in itself, evidence of the inhibitory action of the extracts on development of the head.

A more serious objection to use of the dividing line must be mentioned. Development of the nervous tissue was studied with methods permitting measurement only of the head as a whole. However, even when the lower jaw is omitted, there still remains a large quantity of ectomesoderm and other tissues in the part of the embryo denoted here as the "head".

Before this problem is discussed, it can be pointed out that it is doubtful whether any specific chemical method exists for estimation of the amount of embryonic brain tissue in the presence of other tissues on the 4th day of incubation. There is as yet no formation of specific, characteristic substances that are easily identified and measured, *e.g.* cholinesterase (38). This is in contrast to the presence of Hb, for instance, in the blood, which makes its development easier to analyze. As a substitute for specific methods, the dividing line was used as a basis for planimetric determinations of the projected surface of the head, and the total N content of head and body fragments was also determined.

The planimetric method gives the size of the head, *i.e.,* it measures the development of the tissues and their water content. Determination of the total N content gives the amount of proteins produced during growth of the nervous tissue and of the other tissues of the head. The question then arises whether the variations

in the ratio $\frac{\text{Total N head}}{\text{Total N embryo}}$ do, in fact, represent variations in growth of the nervous tissue. To provide an answer to this question, the study of these variations was combined with histologic examination. Analyses of the N content were made in embryos treated with brain extract, normal embryos (controls) and embryos treated with other extracts than those of brain. Histologic examination was, however, made of only a limited number of embryos.

Histologic examination showed that the part of the head most often subject to abnormal development was that containing the smallest amount of mesoderm, *i.e.*, the region of the 1st and 2nd cerebral vesicles. Moreover, the mesoderm of the body was always well developed, and the blood and cardiac tissues could even be hypertrophied. The curves for benzidine-peroxidase activity in embryos treated with brain extracts were thus shifted towards the highest values (*cf.* Figs. 6 and 7), and development of the heart was often abnormally rapid. Good development of the body mesoderm combined with defective development of the cerebral vesicles is an indication that the brain tissues have been specifically inhibited by the brain extracts. Consequently, when this has been demonstrated, the ratio of the N content of the head to that of the body can be used as a simple measure of brain inhibition.

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The treated embryos with a normal appearance had a low quotient of $\frac{\text{N head}}{\text{N embryo}}$. It can be inferred from Fig. 10 that this quotient was 0.39 for the untreated embryos, and 0.34 for those treated with brain extracts. The corresponding figure for embryos treated with fraction D of brain extract (obtained by electrophoresis) was 0.335.

The results obtained with the planimetric method have not yet been verified by statistical analysis, but this has been done with respect to the ratio $\frac{\text{N head}}{\text{N embryo}}$. The regression line of the variations in the ratio $\frac{\text{Total N head}}{\text{Total N embryo}}$ was calculated as a function of development in the series of embryos treated with brain extract, and in the controls. An analysis of variance was made. The number of treated embryos included in this calculation was 127, and the number of controls 61. The treated embryos were not grouped into those with visible pathologic features and those with a normal appearance, but the whole population was taken into account. This corresponds to the last column of the histogram in Fig. 10. The material used for the statistical analysis was larger than that serving as a basis for this figure. Development of the embryo, ranging from stage 20 to stage 24, was taken as the measure of time. It can be seen that embryos at stage 22 were the most numerous. The mean quotient was found to be 34.2 in the treated embryos, and 39.9 in the controls.

The difference between the level of the regression lines is highly significant

($P < 0.1$ per cent). This result is important, since the measure of total N is directly proportional to the amount of tissue it is desired to determine.

It seems reasonable to draw the following conclusions from the experimental findings described, as well as from their qualitative and quantitative analysis. The brain substance of both chicks and adults contains one or more factors specifically inhibiting growth of the nervous system during its formation in the embryo. These factors act at the moment of regression of the primitive streak of the blastoderm. The question then arises of the moment at which these factors appear in the donor. This matter will be discussed in section V (see p. 189).

Development of the brain is dependent on the development of many structures. There must be harmony between these forms of structure, not only with respect to space, but also with respect to time. "Component events whose timing or dosing is out of time with the general plan disturb, and often break up, the whole performance." (Weiss 1939, p. 482) (48). If the development of any one of them fails to take place, is delayed or is too advanced, the totality suffers. A disturbance of the nervous system may be the result—apart from mechanical disturbance of morphogenetic movements—of an excessive as well as of a defective production of certain structural units. Is it then possible to classify the present inhibition by brain extracts as homologous inhibition of the production of nervous tissue? Analysis of the ratio $\frac{\text{N head}}{\text{N embryo}}$, complemented by the histologic analyses, indicates that this is, in fact, the case.

The experiments of Detwiler, cited by Weiss (55), as well as personal observations, have shown that one anatomic part of the central nervous system can develop even if other parts are absent. Thus, in experiments with other substances than brain extract, embryos with an abnormally developed head had a normal ratio of $\frac{\text{N head}}{\text{N embryo}}$. In four embryos at stage 21 showing various anomalies of the head after injection of RNA*, the average quotient was 0.36, with a range of 0.31 to 0.40. In normal embryos, the average figure is 0.39. In embryos treated with brain extract, the figure may be as low as 0.27 when qualitative deformation of the head is present (based on the values in Fig. 10). It is true that four determinations of the ratio of total N is not a large sample, but in the present case it may suffice to show that the quantity of proteins formed in the head may be normal, despite morphogenetic anomalies.

It must be pointed out that the abnormalities caused by injection of brain extract do not seem to be particularly characteristic. They are of the kind occasionally found in eggs treated with extracts of mesodermal tissue, and sometimes even in nature. The number of embryos showing anomalies in development of

* Although this study does not form part of the present investigation, it can be mentioned that no abnormalities were observed in the great majority of embryos treated with RNA from 12-day-old whole embryos, but their general development was slightly accelerated.

the head is, however, characteristic, *i.e.*, it amounts to about 40 per cent when brain extract has been injected, as compared to only about 5 per cent in other cases. The figure of 5 per cent also applies in embryos with blood abnormalities, in natural populations and in populations treated with brain extract. It seems, in fact, to comprise the usual quota of animals having a certain abnormal organ at the beginning of differentiation, and when dealing with large populations.

In many cases it was observed that the folds of the neural plate were incompletely fused (*cf.* Fig. 11). This is a well-known phenomenon. It is related to exogastrulation and, like this, may be produced by many substances (18).

Omphalocephaly.—Cases of omphalocephaly were relatively uncommon. Consequently, there is no reason to discuss at any length the causes of its appearance in experiments with brain extracts. This anomaly has been studied by WOLFF (57), who attributed its cause to hypotrophy of the head. It is possible that omphalocephaly may be a manifestation of inhibition of development of the head by brain extracts.

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Analogous results obtained by serologic methods have been reported recently. GLUECKSOHN-WAELSCH (1957) studied the effect of maternal immunization against organ tissue on embryonic differentiation in the mouse. She concluded: "The injection of brain emulsion into females of the DBA/1 strain was followed by abnormalities of the developing nervous system in 8—9 % of subsequently conceived embryos." (Suppression of nervous tissue differentiation, microcephaly, and abnormalities of closure of neural folds. In controls—heart-injected mothers —no nervous-system abnormalities were observed in embryos.) "... It seems most likely that maternal antibodies are responsible for the nervous-system abnormalities of the embryos" (11).

A number of experiments with many series of eggs have shown that brain extract in contact with the blastoderm of the chick inhibits the normal formation of the brain or its parts. The extract has no action on the rest of the body.

III. EYE

This organ is composed of a number of parts which emanate from different regions of the neurula. It is therefore necessary to investigate whether the extracts influenced the eye in its entirety, or had only an elective effect on one or other of its parts—optic vesicle or lens. It must be borne in mind that regulating phenomena might have occurred, and caused a readjustment between the parts (*cf. e.g.* HARRISON 1929, BALLARD 1939). (Cited by Twitty, 46.)

The extracts injected were not total homogenates of the eye; the lens having a hard consistency could not be ground by normal means. They therefore consisted principally of the humours, the retina and the tela choroidea. This does not, however, imply that the extracts were wholly devoid of an expected anti-lens substance. In the normal eye, the regenerative capacity of the iris epithelium is held in check by an inhibitory influence, apparently chemical in nature, exerted

by the resident lens (TWITTY 46). It is highly probable that the extracts used in the present experiments contained a small quantity of this substance, even if the lens was difficult to homogenize.

Qualitative evaluation of the effects on formation of the eye is easy, in view of its large size. It is true that the ratio $\frac{\text{Diameter of head}}{\text{Diameter of eye}}$ is not a very precise measurement, but the differences observed between treated and normal embryos were great. The action of the eye extract on eye development is evidently distinct, but the influence on lens development is less clear. Cases were observed in which development of the lens was normal, whereas the optic vesicle was extremely small (Fig. 16 B). Such cases were, however, rare. Although the influence on the lens was less clear than could have been expected *a priori* from the results of WASCH & SATO cited by Twitty, this was probably due to the incomplete homogenization of the lens. It is nevertheless possible that, in these experiments, it was a question of homologous inhibition of formation of the embryonic eye by substances contained in the adult eye.

IV. HEART

The results of the present experiments may sometimes be misleading. Thus, cases in which all organs—with the exception of the heart—developed normally were not sufficiently clear to permit a correct diagnosis. They showed slight retardation of growth, and some difficulty for the cardiac tube to differentiate into distinct chambers (Fig. 17), the action of heart extracts on other mesodermal parts being distinct, *i.e.*, the somites were small in most cases, and the blood was pale or absent. Similar inhibitory effects of heart extracts have been reported in the literature. Thus, when chick blastoderms were cultivated *in vitro* on different substrates, the presence of heart extract inhibited formation of the heart; it also inhibited normal development of the axial organs (SPRATT 1954) (43.)

The anti-heart substances may manifest themselves much more easily under the conditions of more direct contact offered by organ culture and by tissue culture. These cultures are easily done; as early as 1928, OLIVO showed that the cardiac primordium of the chick embryo has a great ability of self-differentiation in culture *in vitro* (cited by Copenhaver, 5). Analogous results were obtained in the present investigation; thus, numerous experiments showed that the contractile tissue of the chick embryo forms very readily. FINNIGAN (10), studying erythropoiesis in embryos of salamanders, observed the same phenomenon—formation of pulsative vesicles of vascular nature. These vesicles formed even more easily than the blood islands. The contractile heart tissue evidently has a great facility of development. Consequently, it is not surprising to find this tissue as a regular component of the clumps of tissue that occasionally developed after injection of brain extract, for instance. "The "humps" can therefore be used to demonstrate the specific inhibition produced by heart extracts. In the present ex-

periments, these complexes or "humps" could be divided into two categories, *i.e.*, those containing pulsative vesicles, and those lacking such vesicles.

Table II shows that the number of humps containing pulsative vesicles was much larger (3.7 times) in eggs treated with brain extracts than in eggs treated with heart extracts. Heart extracts thus exerted an inhibitory action on development of contractile heart tissue, irrespective of whether development of the whole embryo was organized or chaotic. Experiments made by different workers, and with various methods, thus lend support to the theory of homologous inhibition in the case of cardiac tissue, as well as in blood and in nervous tissue.

Another phenomenon that can be considered in this connexion is the reverse of inhibition, *i.e.*, promotion. In the present experiments on inhibition of blood formation, the controls consisted both of untreated blastoderms and of blastoderms treated with brain extracts. The result was somewhat surprising, in that injection of brain extract was generally found to have promoted blood development (*cf.* Fig. 7). The heart tissue was also hypertrophied, even in embryos with morphologic anomalies of other organs.

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The section through the head and body of an embryo shown in Fig. 21 illustrates such cardiac hypertrophy after injection of brain extract. Several curvatures are present, but the embryo is otherwise well developed on the whole. The 3rd cerebral vesicle, auditory vesicle and spinal medulla can be identified, in addition to a well developed forelimb. Examination of the whole series of sections shows specific inhibition of brain development by brain extract; the 1st and 2nd cerebral vesicles have failed to develop. A remarkable feature is the presence of an extra heart; moreover, the total mass of cardiac tissue seems to be greater than in a normal heart at the corresponding stage. Such an extra heart was never observed in the experiments with extracts of mesodermal tissues. The two hearts are not anatomically related; the anomaly may have arisen on the grounds of mechanical disturbance of the normal fusion of the paired heart rudiments. However this may be, an abnormally abundant formation of cardiac tissue has ensued; this is probably to be ascribed to one or even both of the following causes. (1) The brain extract may have had a promoting action on the formation of cardiac tissue, and/or (2) each half of the heart, separated from the other half, has not been subject to the inhibiting action of the other half, and has developed excessively. A phenomenon of this nature is related to the results of the experiments described here.

In cases in which injection of brain extract did not bring about hypertrophy of heart and blood, development of these structures was good, even if the embryos were severely damaged ("humps"). The results were thus exactly the opposite of those with heart extracts. Fig. 18 shows some isolated pulsative vesicles ("humps") formed in the experiments with brain extracts. Some of these vesicles have a large functional heart, connected to a fairly abundant system of blood vessels (*d*). Occasionally, somites and/or limbs have been formed (*c*).

The facts presented give reason to believe that brain extract contains substances which promote mesodermal formations. Alternately, they may indicate that the non-inhibited tissues develop excessively at the expense of the inhibited trend of

development. A competition phenomenon that is probably related to the competition between the different trends of development in the sea urchin egg has been summarized in RUNNSTRÖM's concept of the double-gradient system. It can be pointed out that it is normal for the volume of the blood and the volume of the heart to vary in the same direction, *i.e.*, adaptation (see *e.g.* LANDAUER's (21) analysis of the frizzle fowl).

Although heart extracts have a generally inhibitory and toxicaction—leading in most cases to formation of "humps"—they also seem to act electively and to inhibit the formation of contractile cardiac tissue. In addition, they appear to have a promoting influence on formation of mesodermal tissue.

V. INFLUENCE OF AGE OF DONOR

In the present investigation, studies on the influence of the age of the donor were confined to the use of brain extracts.

The homologous inhibitory action seems to appear on about the 5th or 6th day after the beginning of incubation. This is the stage at which various characteristic proteins, lipids and enzyme activities appear in the central nervous system. If proteins characteristic of adult tissues are already present in the blastoderm at this stage, they can be detected only by very sensitive serologic methods, and only in cultures EBERT (7). The classical immunochemical methods with *in vitro* extracts do not give any marked precipitates before the 6th day (BURKE 1944) or even the 9th dag (GRUNWOLT 1949). Cited by EBERT (7).

The parallelism between appearance of the inhibitory activity and specialization of the embryo is also apparent on the anatomic level; the grey and white cerebral substances develop rapidly between the 4th and 8th days, and then reach a high degree of differentiation. The general architecture of the brain becomes discernible at this stage, and the cerebral hemispheres soon develop.

The inhibitor seems to appear at a very early stage, and its action becomes increasingly marked as the chemical, histologic and morphologic traits make their appearance. The fact that the inhibitory activity arises very early during formation of a new tissue was also observed by SATO (1935), in experiments on regeneration of the lens. Cited by Twitty (46).

VI. NATURE OF THE HOMOLOGOUS INHIBITORS

The inhibitory activity was found to be present in the red corpuscles, whereas the serum had no such activity. Various preparations derived from blood were tested. When solutions of cock Hb that were not too toxic were injected, no specific action on blood development could be noted. An inhibitory effect was, on the contrary, obtained with the residue of blood proteins, after removal of the Hb by precipitation with ammonium sulphate.

The inhibitory activity of blood and brain extracts was not suppressed by dialysis. This indicates that the inhibitors have a relatively high molecular weight.

As far as blood is concerned, this observation is further supported by the fact that extracts of eye had no inhibitory action on blood development, since these extracts consist mainly of aqueous humour, which is a dialysate of blood (ROCHON-DUVIGNEAUD 30).

Studies of the sensitivity to heat showed that the inability of the inhibitors to pass through dialysis membranes is associated with great thermolability (*cf.* Table VI). These two properties of the inhibitory substances were also observed by SAETREN (1957) in regeneration experiments. In the rat, the growing liver and kidney produce thermolabile, non-dialyzable substances, which exert a specific inhibitory action on growth of the homologous organ. The inhibitory action must thus be due to high-molecular substances which are easily denaturated by heat. SHAVER (42), in experiments on Amphibians, showed that inhibitory activity is obtained with the microsomic fraction of brain extracts.

These results are not consistent with the theoretical calculations of WEISS & KAVANAU (56), which seemed to indicate that the inhibitors have a low molecular weight. It is, however, possible that substances of high molecular weight can penetrate the membrane of the living cell, *e.g.* by the mechanism of pinocytosis. Moreover, it is not unlikely that two groups of homologous inhibitors exist, namely, proteins and dialyzable, low-molecular substances.

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Experiments with organ extracts showed development of the homologous organ to be inhibited by about 40 per cent. The action of the extracts might reflect both the content of promoting and inhibitory substances and their relative proportion, as well as their relative rate of penetration into the cells.

It was therefore considered of interest to separate the promoting and inhibitory substances. Since the templates might be related to the microsomes and RNA, the action of the nucleoprotein fraction (NPF) of different organs was tested. KUTSKY (19) tested systematically the action of different cell fractions from 12-day chick embryos on chick heart fibroblasts *in vitro*. He found that the growth-promoting factors remained in the fractions of microsomes and mitochondria, but were partially dialyzable. The core after dialysis was a mixture of nucleoproteins containing about 10 per cent of RNA, but RNA from 12-day-old chick embryos proved to be devoid of action on growth of the heart, cultivated *in vitro*. When the NPF fraction was tested, it was, on the contrary, found to have a strong growth-promoting effect. The NPF of cartilage had a stronger action than the NPF of brain, when tested on the heart fibroblasts.

It is of interest for studies on development to ascertain whether the NPF of different tissue has a characteristic action on the growth of *homologous* tissue. This question is easiest to study on whole organisms. The NPF of different tissues was therefore tested on chick embryos *in vivo*. The NPF of whole embryos, brain and blood was tested on blastoderms. An effect on general growth of the eggs was produced, development always being harmonious. The NPF of brain incited not only growth of the brain, but also that of mesodermal structures. The meso-

dermal NPF enhanced growth of the brain, as well as of the whole body.

According to the aforegoing results, the NPF of brain and blood, as well as of extracts of the whole body, contained growth-promoting substances, but did not act as templates in the sense of WEISS, since they did not electively promote growth of homologous tissues. The brain NPF had a stronger action than the NPF of the whole embryo. KUTSKY *et al.* found the contrary, but the total body NPF used in the present experiments had been stored for a longer time than the brain NPF, and it is known that NPF must be used when fresh, since its action becomes weaker with time.

The NPF of brain had a weak (21 per cent) selective inhibitory action on development of the head, whereas the NPF of mesodermal origin had no selective inhibitory action on either head or body development. The toxicity of brain NPF might be due to its contamination by some homologous inhibitors. It is likely that such contamination may have occurred, since the NPF was not precipitated repeatedly, as recommended by KUTSKY.

The growth-promoting action was most apparent when the NPF was tested on late neurulae (stages 8 and 9), at which stages the inhibitory substances had no effect.

The residue obtained after precipitation of the nucleoproteins by streptomycin was denoted tentatively as a non-nucleoprotein fraction (ONPF). Neither the chemical nature of this fraction, nor its possible content of traces of nucleoproteins, has yet been determined exactly. It has, however, been found to contain non-dialyzable, thermolabile substances which specifically inhibit development of the organ homologous to that from which it is derived.

The results of this preliminary study can be summarized as follows. The homologous inhibitors seem to be localized to certain fractions of the tissue extracts. The nucleoprotein-containing fractions have a somewhat unspecific growth-promoting action on the embryo, whereas those practically devoid of nucleoproteins seem to contain the homologous inhibitors.

VII. COMPARISON WITH OTHER EXPERIMENTS ON THE PROBLEM OF DIFFERENTIATION BY HOMOLOGOUS INHIBITION

It is beyond the scope of this paper to review all the experiments which indicate the existence of a mechanism of homologous inhibition during development. The discussion will therefore be confined to certain types of experiment of importance for the understanding of homologous inhibition.

Prospective Areas and Differentiation Potencies

Many of the classical investigations in experimental embryology have dealt with the development of presumptive organ areas and their fate under normal conditions, or after separation from their natural environment. It is now a well-established fact that prospective areas are not primordia; the areas of histogenetic

potencies are much larger than prospective areas, and partly overlap each other (*e.g.* HOLTFRETER 1936, RUDNICK 1948) (16, 36). This fact, as well as numerous experiments on regeneration or on tissue cultures (*e.g.* HARRISSON 1918, LOPASHOV 1935), show that, during differentiation, the tissues act on each other (12, 25). Moreover, the most active centres of differentiation inhibit the possibility of the adjacent areas of actualizing their potency to differentiate in the same direction.

We are thus in a position to envisage the young, still undifferentiated cells, with a high morphogenetic potentiality, as being under the influence of groups of physico-chemical factors acting in opposite directions. Some of them promote specialization in certain directions, whereas others, on the contrary, inhibit specialization in a direction already occupied. These are the inductors and inhibitors of differentiation, respectively. As soon as a tissue is formed, or even is in a state of formation, it yields one or several inhibitors which arrest the normal process in cellular groups where these processes are slower, or start later. They are therefore obliged to specialize in other directions. Thus, by successive elimination, a histologic and organic diversity is produced.

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Regeneration

The chief biological difference between an egg and a blastema of regeneration is that the egg is a closed system, whereas the blastema is an open system, *i.e.*, a system depending on the rest of the body of the operated embryo. The biological systems which it has been tried to create experimentally are situated halfway between the closed system represented by a normal chicken or frog egg, and the open systems represented by a blastema. An adult tissue or an organ extract is introduced into the egg. As a result, the blastoderm—which, normally, is *not* submitted to the action of any adult organ, apart from any adult tissue compounds which may be present in the yolk—is under the influence of the action of *one* adult tissue or tissue extract. The blastema of regeneration, on the other hand, undergoes the biochemical action of *several* adult tissues and organs.

The neoblasts of the blastema have to adapt themselves to the biochemical influence of already existing tissues. The blastoderms of the injected eggs also have to adapt themselves to the extracts of organ introduced into the system. It seems of interest to compare these phenomena of adaptation. In the experiments made in the present investigation, the potentialities of the blastoderms were limited by the inhibitory action of the tissue or tissue organ which had been introduced into the egg, this inhibitory action being restricted to formation of the homologous tissue or organ.

Similar results could be noted in the experiments on regeneration of *Planaria* made by BRONDSTED (2). A differentiating head of *Planaria* thus produced anti-head substances which checked development of other heads from tissues with head-forming capacity in the anterior region of the animal. The only difference between these tissues and the tissue which normally forms a head was that they

differed with respect to the rate of head formation. In the tissue where differentiation was most rapid, the head formed produced anti-head substances which prevented the neighbouring areas from forming head structures.

The existence of anti-head substances was also demonstrated in the experiments of SANTOS, cited by BRØNDSTED. Segments of the head and its ganglions were implanted in the cavities made in the body of the host. When these implantations were post-pharyngeal, the graft was absorbed under the influence of the host's head.

LENDER (1956) (24) found that when decapitated *Planarias* were placed in a bath containing head extract, regeneration did not normally occur; thus, the extract inhibited formation of the brain. When the same experiment was made with tail extract, it resulted in animals with normal heads.

The phenomenon denoted as Janus head is of interest in this connexion. When a cut is made in both the anterior and posterior parts of the *Planaria,* a short fragment of tissue is obtained, capable of regenerating the head on either side of the animal. The question then arises whether this phenomenon is contradictory to the results of experiments seeming to demonstrate differential inhibition.

A prerequisite for obtaining a Janus head is that the two cuts are made simultaneously, and that they are close to each other. The relative rate of formation of tissue, and the chances of conveying the inhibitor produced to a region not yet differentiated, but capable of differentiating in the same direction, are equal. This may explain the formation of Janus heads.

The phenomenon of Janus head formation does not contradict the theory of homologous inhibition, for, on one hand, the fragment is a short one, and the density of its cephalogenic properties is therefore almost homogeneous. On the other hand, the two cuts being made simultaneously, no high point has had time to set in between the two operations. Thus, the factors of time and special heterogeneity being eliminated, a system is obtained which is both closed and neutral, and in which the inhibitors as well as the inductors are mutually balanced.*

BRØNDSTED (1955), in the conclusion of his review of *Planaria* regeneration, put forward the following hypothesis with respect to differentiation during regeneration:

> "The totipotent neoblasts start at a given level in the time-graded regeneration field: they regenerate head or tail, respectively, until morphogenesis by differentiation of the neoblasts has filled up the lacking biochemical niches; then the neoblasts are inhibited from going further by the serological factors differentiated in the already existing adult tissues."

The role of inhibitors in the regeneration and maintenance of balance between the organs is not limited to invertebrates. Even in vertebrates, we find that the same inhibits the same. If the power of self-inhibition of an adult organ is sud-

* For a survey of other experiments on regeneration in the invertebrates, reference is made to ROSE (34, pp. 370—371).

denly diminished by removing a large portion of it, it regenerates its own tissues, but not another kind of tissue. For example, DETWILER (cit. WEISS 1955) (55) removed some regions of the central nervous system in a young embryo; if the cut spared the half symmetrical with the part removed, regeneration took place, and the embryo developed normally.

The production of blood increases after hemorrhage. Regeneration also follows removal of a large section of hepatic tissue. In the latter case, it is clear that the phenomenon affects the whole organism. If the blood of the operated animal is brought into contact with the blood of a normal animal (parabiosis), the liver of the normal animal starts to develop. The greater the loss, the greater is growth (ROSE 1952, WEISS 1952). SAETREN (40) showed in the rat that both the kidney and liver give rise, when regenerating and growing, to a substance which exerts a specific inhibitory action on the growth of its organ of origin.

The studies of WACHS and of IKEDA (cited by Twitty) on Wolffian regeneration have shown that there are two prerequisites for occurrence of this phenomenon, *i.e.*, absence of the lens and the presence of retinal tissue. Referring to these investigations, TWITTY (46) summarized the situation as follows:

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"On the basis of these results, it was postulated that the behaviour of the dorsal iris is controlled by a secretory balance, existing between the lens and retina. According to this concept, a retinal secretion conducive to lens formation is normally counteracted by another originating from the resident lens . . ."

It can be concluded from all these experiments on regeneration that specific inhibitory agents produced by differentiation exist, in addition to inductive agents.

Grafts and Injection of Extracts

A number of experiments are reported in the literature which apparently contradict the theory of homologous inhibition, namely, those of WEISS (1950, 1953), EBERT (1954) and WALTER (1956) (52, 54, 9, 47.)

Thus, WEISS (1950) observed that incorporation of a liver fragment from a 6-day chick embryo in the vascular area of a 4-day embryo caused a specific reaction by the homologous tissue of the host. One week after operation, the liver of the host was 40 per cent heavier than that of the controls. The same author (1953) injected kidney extract intravenously into the embryonic blood vessels, and counted the mitoses in the kidney of the host. The number had increased by 50 per cent in comparison with the normal figure.

EBERT transplanted fragments of spleen of an adult chicken into the chorioallantoic membrane of a 9-day embryo, and noted an increase in volume of the homologous organ of the host. Quantitatively, the results were found to depend on the age of the donor. The effect was clear with a 14-day donor (time of incubation), and increased with age.

Studies on Homologous Inhibition in the Chick Embryo

WALTER injected adult tissue homogenates into 9-day-old chick embryos, and analyzed the influence of this treatment on formation of similar embryonic proteins with the use of tracer methods. He concluded: "It has been demonstrated that injected homogenates give results qualitatively and quantitatively similar to those obtained on grafting chicken tissue to the chorioallantois." See also MURPHY (26).

In reality, these results obtained with grafts and injections do not contradict the theory of homologous inhibition. Presumably, they have only revealed an additional mechanism which takes part in growth and differentiation. A question of fundamental importance nevertheless arises, namely, under what circumstances do the inhibitory substances disclose their presence, and when does specific promotion of growth dominate?

WEISS has explained the apparent contradiction in the results obtained with organ extracts in various experiments by a theory of templates and antitemplates. These two groups of antagonistic substances have different physico-chemical properties, which permit their separation already at the time of their formation, the templates collecting in the organ, and the corresponding antitemplates being dispersed over the whole organism.

In a discussion of these problems, it must be borne in mind that the injection or graft may have been made at a very different stage of development. The conditions thus differ greatly in experiments in which the grafts have a growth-promoting effect, and those in which the effect of the extract is inhibitory. The age of the host differs in the relevant cases, which is important in view of the fact that the physico-chemical state of an organ varies appreciably with age. Actually, when a young blastoderm is treated with an extract, the donor is entirely different to that in experiments with injection or grafting into an embryo at an advanced stage of development. Therefore, from the physico-chemical point of view, the same is not treated by the same. In this connexion, "the same" denotes only the same direction of development from the presumptive area in the blastoderm to the mature organ. Consequently, no comparison is permissible between experiments in which the actual organs or organ extracts are brought in contact with a homologous organ formed in an embryo, and those in which the extracts are injected beneath presumptive areas at the moment of formation of the primitive streak in a blastoderm (or in a late gastrula in the frog).

When embryos in early stages—*e.g.* blastoderms—are treated, homologous inhibition may operate in the process of differentiation (specialization), whereas in differentiated embryos the growth processes are affected. In the former case, there may be homologous inhibition of the process of differentiation. In the latter, specific promotion or inhibition of growth may occur, the effect depending on the relative concentration of substances with opposite effects, *i.e.*, templates and antitemplates. It is as yet too soon to discuss whether the presumed inhibition of

differentiation at early stages is due to the same agents which affect the growth at later stages.

Organ Anti-Sera

In view of the similarity of the results, it is of great interest to compare experiments in which the tissue or tissue extracts have been brought in contact with embryos during development, and those in which blastoderms and embryos have been treated with organ anti-sera. WEISS (1939, 1947) injected anti-sera to organs of adult chickens into 4-day embryos. Despite a general retardation of growth, the embryos which developed showed a considerable increase in weight of the organ homologous to the anti-serum injected (49, 51).

EBERT (1952) extracted and cultured chick blastoderms on media containing normal serum (controls) or organ anti-sera (anti-heart, anti-brain and anti-spleen). Development of the embryos varied with the concentration of the anti-serum. At a high concentration, phenomena of degeneration occurred, with formation of cell clumps. At critical intermediate concentrations, relatively specific effects appeared, the action of anti-heart and anti-spleen sera differing sharply from that of anti-brain serum. The former affected mainly mesodermal elements, whereas anti-brain sera affected chiefly nervous tissue. Only anti-heart sera influenced heart development, and in these cultures the heart was absent or its development was extremely retarded. At weak concentrations, the effect of the anti-sera was confined to a delay in general growth, sometimes with formation of dwarfs. The conclusions drawn by EBERT are the same as those in the present investigation. He stated (p. 71):

> "There is no reason to believe that those antibodies produced against specific tissue antigens are not active in high concentrations, but rather that their specific effects are masked by the more generalized inhibition of blastoderm. Specific effects at intermediate concentrations, then, are due to the fact that the non-specific antibodies are diluted out of their activity at greater dilutions."

The results of treatment of eggs at early stages of development obtained by EBERT and by GLUECKSOHN-WAELSCH on the one hand, and those obtained in the present experiments on the other hand, are to some extent analogous. This is despite the fact that the active agents were antibodies in the former case, and tissue extracts in the latter. In both cases the influence may have taken place at the site of synthesis of specific proteins, although the primary mode of action can be postulated to differ in the two cases.

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General Conclusions

On the basis of a large number of experiments, using a variety of tissue extracts and different methods of diagnosis, it seems permissible to draw the following conclusions:

It is highly probable that the adult tissue contains several groups of antagonistic agents, the balance of which ensures development of the different tissues and organs in harmonious proportions.

Among these factors, one group can be denoted as homologous inhibitors. Their presence has been demonstrated in extracts of brain, eye, blood and heart. These homologous inhibitors seem to be localized to certain fractions of the extracts, which are non-dialyzable and thermolabile, and contain nucleoproteins among other substances.

In tissue extracts from blood and heart, homologous inhibition is sometimes accompanied by generalized inhibition of growth. In such cases, the action of the homologous inhibitors can be unmasked by fractionating the extract. The extracts also contain general growth-promoting factors, which appear in the fraction with a high nucleoprotein content.

The existence of homologous inhibition also seems to be shown by the experiments made by different workers on the problem of regeneration, chiefly in invertebrates. The results of a number of studies, mainly on vertebrates, are contrary to those of the present investigation, since they have disclosed homologous promotors of growth. The conditions nevertheless varied greatly with respect to the physiologic age of the experimental material in the relevant cases. The authors in question used already differentiated embryos, whereas young blastoderms were used in the present experiments.

General Summary

The object of the present investigation was to obtain evidence of the validity of the theory of homologous inhibition, by means of a large series of experiments, amenable to statistical analysis.

MATERIAL AND METHODS

Material.—The hen's egg (white leghorn) was used into. Injection was made the blastoderms at stages 5—6 in LILLIE's classification. Altogether about 2,000 blastoderms were used, apart from the controls.

Tissue extracts (blood, brain, eye, heart and whole body) were prepared by homogenization in isotonic Tyrode solution, followed by sterile filtration (GM5 Jena filter). Preparation was performed at 0—4° C.

Injection was made into the germinal cavity against the inferior surface of the

blastoderm, the needle being driven in at the margin of the germinative disc. This ensured close contact with the presumptive organ area.

Diagnosis.—After 4 days' incubation, a *qualitative examination* was made of the general state of the embryo. It included a study of the shape of the head, the cerebral vesicles and sensory organs, spinal medulla, cord somites, extremities and blood, as well as of the state of the heart and the contractility of the cardiac tissue. *Semi-quantitative examination* consisted of colorimetric determination of the hemoglobin content (TALLQVIST's scale), and planimetry (brain). *Quantitative determinations* were also made, *i.e.*, benzidine-peroxidase activity of the blood, and the mass of different organs expressed as their total nitrogen content.

RESULTS

Blood

Development of the blood is found to be more or less inhibited by injection of blood extracts. This applies not only to the percentage of hemoglobin (Hb) but also to the total quantity. The probable explanation is considered to be that the blood inhibitors also act on formation of the vessels whose origin is closely related to that of the embryonic blood.

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Brain

Specific abnormalities of the brain are observed in 40 per cent of the cases in which brain extract has been injected. Qualitative examination shows variable effects. Thus, in some cases the head is small in proportion to the body, and division into five ventricles has failed to occur. In some cases the folds of the neural tube are incompletely fused.

Planimetry discloses large individual variations in size of the head at a given stage, not only in treated embryos, but also in normal populations. A tendency to lower values is, however, found in the treated embryos.

Determination of the variations in the ratio $\frac{\text{Total N head}}{\text{Total N embryo}}$ at stages 20—24 shows a characteristic inhibitory action on development of the head by injection of brain extracts. An analysis of variance shows the difference between the level of the regression lines in the treated embryos and in the controls to be highly significant ($P < 0.1$ per cent).

Eye

Injection of eye extract is found to inhibit formation of the eye in 50 per cent of the cases. The lens is not invariably inhibited. Injection of an extract of medulla oblongata into the controls shows no action on the 1st or 2nd cerebral vesicles, nor on the eye. The influence on the 3rd cerebral vesicle is questionable.

Studies on Homologous Inhibition in the Chick Embryo

Heart

Extracts of heart tissue proved to be highly toxic. As a rule, they are found to have a generally inhibitory effect on formation of mesodermal tissue. They also markedly inhibit development of contractile tissue. In the controls, injection of brain extract seems to have a promoting effect on formation of heart and blood.

Influence of Age of Donor

Extracts from 4-year cocks, 6-month hens and 1-week chicks are found to have a comparable effect. On the other hand, the homologous inhibitory effect of brain extract from donors aged 5—6 days is considerably weaker than that from 12-day-old donors. It is therefore concluded that the inhibitory action of brain tissue probably increases markedly between the 5th and 12th days of incubation. It is pointed out that a characteristic feature of this period of embryonic life is the rapid development and differentiation of the brain and its accessory structures.

Nature of Homologous Inhibitors

An account is given of a preliminary study of this question. It indicates that the inhibitory substances have a relatively high molecular weight, since they are non-dialyzable and thermolabile. It is also probable that they are not nucleoproteins, since the inhibitory action of fractions with a high nucleoprotein content (NPF) is found to be weaker than that of fractions practically devoid of these substances (ONPF). Moreover, the former fractions are found to have a generalized growth-promoting effect.

DISCUSSION

The aforegoing results are discussed. It is pointed out that large series of experiments have been made, and that each series contained a large number of individuals. Moreover, a variety of extracts have been used, and both qualitative and quantitative diagnostic methods have been applied. All these factors contribute to enhancing the value of the results obtained. It is true that the studies on the nature of the homologous inhibitors have not yet led to any exact results. They have nevertheless made it possible to distinguish between the inactive fractions of the extracts, and the fractions which have a stronger inhibitory action than the extract from which they have been prepared. This confirms the preceding results regarding the existence of homologous inhibition.

The literature still contains few articles on the problem in question. Experiments have, however, been made by Rose on amphibian eggs of the same physiologic age as the chick blastoderms used in the present investigation. They led this author to elaborate the theory of the mechanism of differentiation by homologous inhibition, but the experiments were not large in number. Numerous experiments have been made on the regenerative ability of different organs in both vertebrates

and invertebrates. Their results, even if they do not always directly confirm those obtained in the present investigation, nevertheless point in the same direction. Consequently, they encourage further research along the same lines.

The results of certain experiments on the chick are, however, found to be at variance with those obtained in the present investigation. It is suggested that the possible cause of this discrepancy is the influence of the age of the host at the moment of intervention. This is because, in such cases, the course of development is dependent on the stage of development of the embryo at the moment of injection or grafting. Consequently, these results do not invalidate those obtained in the present experiments.

CONCLUSIONS

On the basis of the present experiments, and of the data in the literature, it is concluded that a mechanism of differentiation by homologous inhibition does, in fact, exist. This mechanism takes its place among other factors of differentiation and of balance in the mass of tissues under formation, respectively, at a later stage of adult tissues.

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Addendum

While this work being translated to english and printed three publications on the same subject have appeared. Braverman (october 1958) and Clarke (1959) get the same results as have been reported in this work. Croisille used about the same methods as Weiss and completed his findings. See discussion (p. 195).

The most important of my preliminary results were reported in Rose's review: cellular interaction during differentiation (1957). (34)

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On the ontogeny of the chondral skull in Characidae, with a discussion on the chondrocranial base and the visceral chondrocranium in fishes

by

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I. Introduction

It is well-known that the large family *Characidae* (or *Characinidae;* Berg 1958) is very interesting in several respects such as the teeth (Rowntree 1906, Eastman 1917 and Monod 1950), the osteocranium (Müller and Troschel 1845, Sagemehl 1884, Boulenger 1897, Eigenmann 1899, Regan 1911 and Gre-

GORY & CONRAD 1938), the visceral anatomy (ROWNTREE 1903) and the phylogeny (SAGEMEHL 1885, BOULENGER 1901, ROWNTREE 1903 and NICHOLS 1928, 1930). However, no investigation has been made as yet on the chondrocranium and its ontogeny nor on the ontogeny of the osteocranium. The present paper will partly deal with the former problem.

As the characids are said to derive from Cretaceous (EASTMAN 1917) such an investigation may be expected to reveal problems of great phylogenetic interest. In order to obtain an explanation of certain problems found by the study of the characids a comparative investigation has been made on other gnathostomian fishes: actinopterygians and dipnoans as well as selachians. Such problems are for example the composition of the chondrocranial base and the homology of the trabecles and the prootic visceral arches.

In this paper the basic work on the characids has been performed on the African characid *Hepsetus (Sarcodaces) odoë,* which belongs to the disjunctive sub-family *Sarcodacinae* (EIGENMANN 1899). However, in special problems (the composition of the cranial base, the visceral arches and the ethmoidal region) also other genera are accounted for. 140 series of alltogether 10 characid species have been investigated and partly reconstructed. The phyletic relationship between the characids has been studied but will be published in another connection. As to the general phyletic problems accounted for in this paper all the characids seem to agree with *Hepsetus.*

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This investigation has been performed at the Zootomical Institute, University of Stockholm. It has been faciliated through grants from "Regnells zoologiska gåvomedel" and "Ahlstrands fond". In order to prepare the figures reproduced in the text, grants have been given by the Swedish Science Research Council. To the board of these foundations and to the Research Council I wish to express my sincere gratitude. I also wish to convey my sincere thanks to Professor emer. T. Pehrson, Professor L. Silén, Professor A. Johnels, Professor emer. E. Stensiö and Assistent Professor E. Jarvik for the time they have given me to discuss many problems of general and special morphological interest. Their help has been most valuable. To my assistent Mrs Gunne Weiss I also would like to give my sincerest thanks for her conscientious work with the illustrations as well as to Mrs Benita Ramel who has had the kindness of reading my manuscript.

II. Material and methods

The embryologic material used in this investigation is kept at the Zootomical Institute and partially prepared by other members of the staff of the Institute. The characid material consists partially of African species brought to Stockholm

by the two Swedish expeditions to Gambia in 1931 and 1950 and partially South-american characids reared in Stockholm.

The material is as follows, grouped according to Berg (1958).

ELASMOBRANCHII		
Selachii		
Squalus acanthias	20 series,	3.75 – 59 mm
DIPNOI		
Neoceratodus forsteri	39 " ,	egg – 52 "
Lepidosiren paradoxa	1 serie,	adult
Protopterus annectens	97 series,	egg – 30 "
TELEOSTOMI		
Actinopterygii		
Polypteriformes		
Polypterus senegalus	10 " ,	6.75 – 37 "
Acipenseriformes		
Acipenser güldenstaedti	6 " ,	7.8 – 19 "
" *ruthenus*	20 " ,	egg – 44 "
" *stellatus*	6 " ,	10 – 21 "
Amiiformes		
Amia calva	29 " ,	6 – 56 "
*Lepisosteiformes**		
Lepisosteus platystomus	33 " ,	6 – 70 "
*Clupeiformes (Isospondyli)***		
Salmonoidei		
Salmo salar	235 " ,	egg – 30 "
Esocoidei		
Esox lucius	69 " ,	8 – 33 "
Osteoglossoidei		
Heterotis niloticus	7 " ,	5.6 – 14.5 "
Cypriniformes (Ostariophysi)		
Characinoidei		
Alestes nurse	9 " ,	7.7 – 22 "
Citharinus citharinus	4 " ,	10 – 14.5 "
Distichodus brevipinnis	5 " ,	10 – 62.5 "
" *rostratus*	4 " ,	14 – 16 "
Hemigrammus ocellifer	17 " ,	2.5 – 6.8 "
Hepsetus odoë	70 " ,	egg – 45 "
Hyphessobrycon innesi	7 " ,	2.9 – 17 "
Nannaethiops unitaeniatus	1 " ,	8 "
Nannocharax ansorgei	1 " ,	15 "
Nannostomus aripirangensis	16 " ,	1.5 – 4.1 "
Perciformes		
Percoidei		
Perca fluviatilis	11 " ,	egg – 9.3 "

* For conveniance *Lepisosteiformes* and *Amiiformes* are in the following account put together into the old group *Holostei* (Stensiö 1932, Westoll 1944; cf. p. 351).

** The old name *Teleostei* is used in this paper as a collecting designation of the enumerated fishes having no tubulae in scales and bones (Berg 1958, cf. Westoll 1944, Nielsen 1949 and p. 351).

In addition to this listed material series of the following fishes have been used as a comparision:

Anguilla vulgaris (2 ser.), *Bothus maximus* (6 ser.), *Chanos chanos* (1 ser.), *Clupea harengus* (6 ser.), *Cobitis barbatula* (10 ser.), *Coregonus lavaretus* (19 ser.), *Coregonus albula* (2 ser.), *Corydoras paleatus* (2 ser.), *Cyclopterus lumpus* (28 ser.), *Etmopterus spinax* (5 ser.), *Gadus merlangus* (2 ser.), *Gaidropsarus cimbrius* (1 ser.), *Galeichthys* sp. (11 ser.), *Gobius* sp. (3 ser.), *Gymnarchus niloticus* (11 ser.), *Leuciscus rutilus* (12 ser.), *Pellonula vorax* (3 ser.), *Petrocephalus brevipedunculatus* (2 ser.), *Phoxinus phoxinus* (2 ser.), *Salmo salvelinus* (100 ser.), *Scomber scomber* (22 ser.), *Siphonostoma typhle* (2 ser.), *Syngnathus acus* (10 ser.), *Trachurus trachurus* (2 ser.) and *Zoarces viviparus* (16 ser.).

Fixation of the Southafrican characids has been performed acc. BOUIN, SMITH (1912) and BODIAN (ROMEIS 1943). The African material had been fixed earlier at the Institute. The fixations used were BOUIN's and SMITH's liquids.

The material has been embedded in paraffin and sectioned in series. As a rule the thickness of the sections has been 4—8 microns.

The preparations have been stained in Azan-Mallory (HEIDENHAIN), TOOT's modification of MASSON's tricromstaining, chromalun-hematoxylin (GOMORY) and acc. BODIAN (ROMEIS 1943).

Graphical reconstructions based on the series have been used to a large extent. Photographs of the sections have served as a basis for the reconstructions.

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III. Remarks on the ontogeny of the chondral skull in Characidae, with special reference to Hepsetus odoë

HISTOLOGY

"The appearance of every cranial component depends upon its earlier development and in order to come closer to an understanding of the cranial skeleton, it is necessary to trace its development as far back as possible. This demand becomes more urgent if one strives to get dates for comparative anatomical and phylogenetical purposes." This statement by HOLMGREN (1943) was originally made, because all descriptions and reconstructions of *Squalus acanthias* published by different authors were so inadequately made that it was impossible to get any satisfactory developmental history of the skull of that selachian. The authors had begun their investigations with the stage "where the first cartilage is developing, paying less attention to those very important developmental processes preceding that stage. In reality the appearance of the first cartilages, the parachordals, is a rather late process in the development of the skull which in its blastematic or mesenchymatic stages has already received the outline which it retains throughout stages much later than the beginning of chondrification" (HOLMGREN 1943).

These principles are valid also for other fishes and for the present time. And we may add: if we want to draw conclusions of phyletic value and to avoid the specific functional adaptations which occur later, we ought to investigate the early ontogeny of almost the e n t i r e h e a d, especially the relations between the cranium and vessels, nerves and muscles. This investigation has been made in that way and with the intentions set forth by HOLMGREN.

The first data of the histogenesis of hyalin cartilage are found in STRASSER's (1878, 1879) investigations of the amphibians *Triturus* and *Salamander*. Later they have been completed and extended to fishes especially by SCHAFFER (1901, 1930), STUDNITZKA (1907) and LUBOSCH (1938). As to the teleosts and the dipnoans the cartilage is said to develop from mesenchyme via a prochondral stage (LUBOSCH). SCHAFFER (1930) has the same opinion but has added two stages, a blastemal stage between the mesenchymal and prochondral stages and a protochondral stage between the prochondral and chondral stages.

In the *blastemal stage* or "Die erste Anlage" the spindelshaped mesenchyme cells have according to SCHAFFER united to a symplasmatic mass, a syncytium, with rounded "stark färbbare ovoide Kerne durch eine spärliche zunächst cytoplasmatische Zwischensubstanz zusammengehalten". The *prochondral stage* is characterized by the blastema being split into its original units, the cells, since these are separating from each other by an intercellular substance. The tissue is, however, still acidophile and apparently homogenous. In the third stage, the *protochondral stage,* the intercellular substance has become basophile and fibrillous by secreting "eine chondromucoide Kittsubstanz". The next link in the histogenesis is essentially tied to the cytoplasm, which grows considerably in volume, whereas the intercellular substance has almost ceased to grow. The tissue has now reached the *chondral stage*. By secretion of a matrix of different thickness and the formation of capsules around the cells and a perichondrium around the cartilage, the chondral tissue then gets its normal hyaline appearance.

As HOLMGREN, LUBOSCH and others have a tendency of using the terms mesenchyme and blastema in a rather unspecified way, it is important to state that SCHAFFER's description is valid also for the fishes investigated by the present author (pp. 205—206).

For this account it may also be of interest to add a few lines concerning the difference between chondro- and osteoblastema and the possibility of demarcating them. The osteoblastema is in late blastema stage characterized by more spherical and strongly stained nuclei. There is, however, an even transition between the two types, and in early blastema stage it has not been possible to detect any differences at all.* The literature does not give any solution of this problem either.

As far as the demarcation of the blastemas is concerned, it should be pointed

* The possibility of using different staining methods was restricted, however, as the material in most cases had been fixed earlier acc. BOUIN and SMITH (p. 206).

out that there are no membranes or other mechanical dividing factors. It is, however, possible to get a satisfactory demarcation line if the blastemas are densely nuclified or surrounded by a different cell type. Both of these circumstances occur in *Hepsetus* and in all teleostomes and dipnoans presented in this paper. On the other hand the outlines can be more diffuse in the selachians, principally owing to a decreasing cell density towards the periphery. In those few cases where the blastema in teleostomes and dipnoans is sparcely nuclified and diffuse, this has been indicated by wavy lines in the reconstructions.

The differentiation of the cells of the neural crest (ectomesenchyme of DE BEER 1947) into visceral cartilage is a problem which has occupied an advanced position in embryology. In fishes DOHRN found already in 1902 in *Torpedo* and GREIL 1913 in *Neoceratodus* a histological difference between ecto- and endomesenchyme.* Later on HOLMGREN (1940) verified DOHRN's findings and in 1943 also those of SEWERTZOFF (1928) in *Acipenser*. It was to some extent possible for HOLMGREN to extend those differences even to SALMO (1943).

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It is very difficult to discern any histological differences between endo- and ectomesenchyme in *Hepsetus*. In *Neoceratodus* and *Acipenser* this difference, however, is quite obvious and of the same general type as in selachians and amphibians, i.e. the endomesenchyme has big yolk globules (Fig. 63) that are not resorbed until after the chondrification has begun; the ectomesenchyme, on the contrary, has only small droplets being resorbed already in the blastemal stage. These distinct differences are of a special interest for the question whether visceral elements may fuse to the neurocranium (p. 301).

DESCRIPTION

Stage I, 3.9—6.5 mm (Figs. 1—5).

The external morphology is still dominated by the big yolk sac. The eyes and the sucking discs (Fig. 1, s.d.) fill a considerable deal of the head.

The internal morphology is dominated by the notochord (ch.), the otic vesicles (ot.v.) and the nasal sacs (na.s.). The cranium is, with the exception of the notochord, almost completely blastemal. Important vessels and nerves are also beginning to differentiate.

Neural cranium

The notochord is the most important supporting element. It is a thick rod extending through the middle of the head up to the anterior margin of the otic vesicles. It almost reaches the hypophysis (Fig. 2, hyp.).

* The terms "mesentoderm" and "mesectoderm" introduced by PLATT (1893—1897) have frequently (at least in anglosaxian literature) been altered into "endomesoderm" and "ectomesoderm", since these terms express the difference in their origin more exhaustively. However, for the same reason as the term "ectomesenchyme" has been found more appropriate than "ectomesodermal mesenchyme" to express cells migrating from the ectoderm (DE BEER 1947, HÖRSTADIUS 1950), the term "*endomesenchyme*" should analogically be used for cells moving out of the endomesoderm.

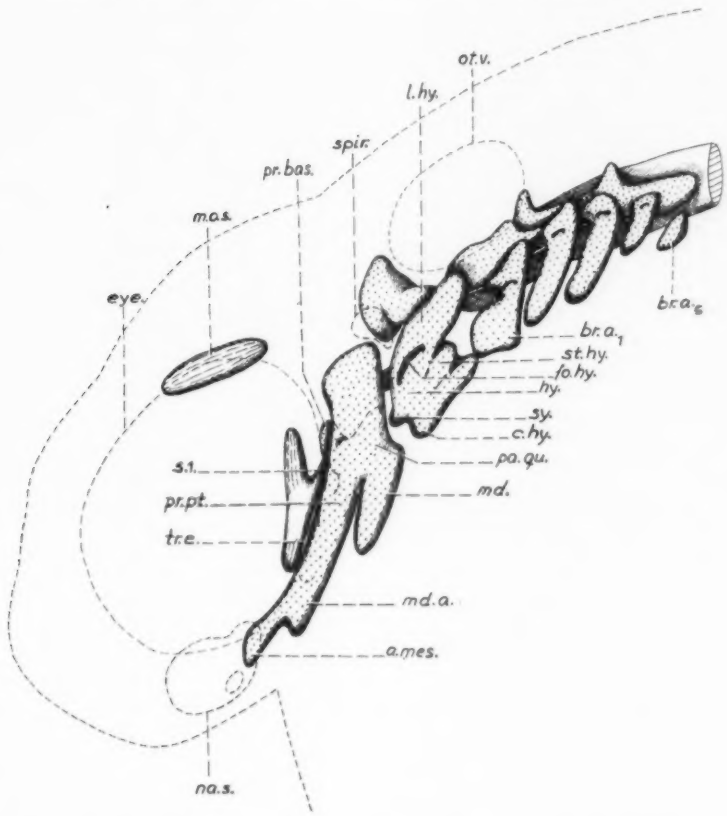

Fig. 1. *Hepsetus odoë*, 6.5 mm. Reconstruction of chondrocranium, lateral view. Mesenchymal and blastemal parts dotted. Muscle tissue sligthly waved lines. Contours of head, nasal sacs, eyes, spiracular vestige and otic vesicle broken lines, sectioned surfaces continuous lines. 100 ×.

The other supporting elements of the cranial base are grouped on each side of the big notochord, i.e. the trabecles, the polar cartilages, the parachordalia, the basiotic laminas and the otic cartilages. All these elements are blastemal.

Each *trabecle* consists of two parts, one laterally (trabecula exterior; Fig. 2, tr.e.), and one medially (trabecula interior; tr.i.) to the carotis interna (c.i.). As initially only the medial part is connected with the polar cartilage, a carotid incissure (c.fi.) is formed. This incissure of the trabecles is noteworthy and seems to be comparable to the one found in *Acipenser* and *Amia* (HOLMGREN 1943; cf. p. 298).

Each trabecle is along its dorsocaudal margin connected with a loose cord of mesenchymal cells (Figs. 1, 3, s. 1), concentrically arranged in a regular manner. This cord is rostrally connected with the primordium of the musculus (m.) rectus

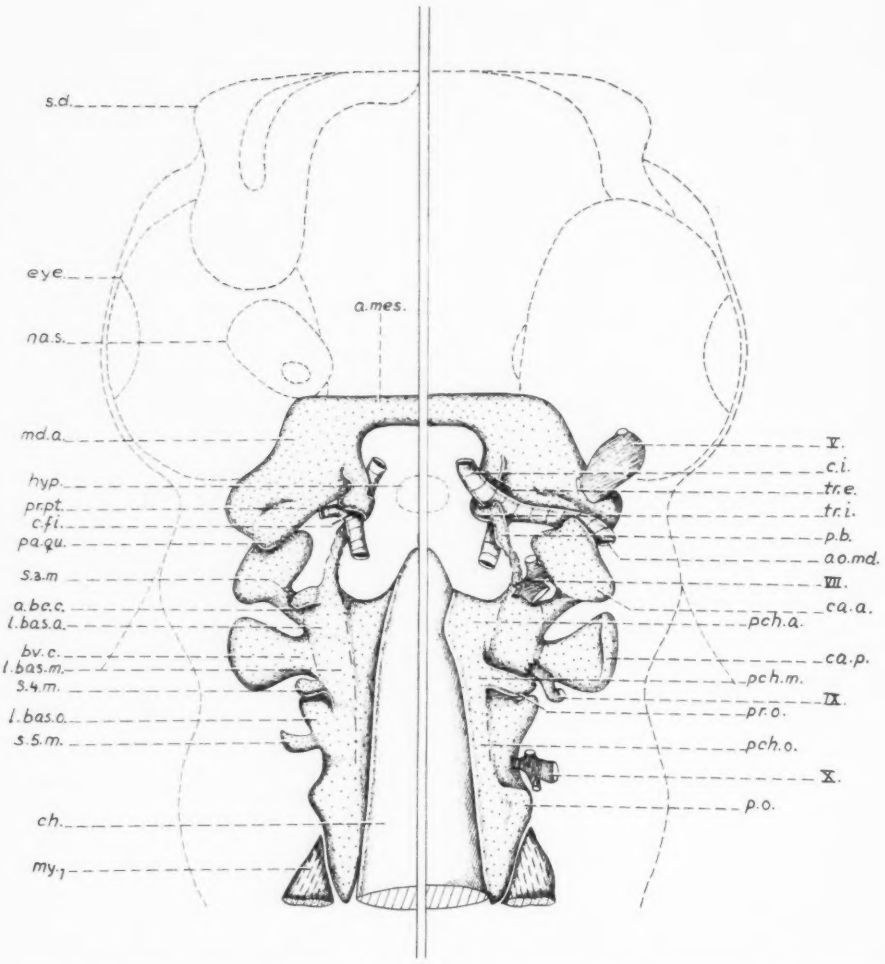

Fig. 2. *Hepsetus odoë*, 6.5 mm. Reconstruction of neural chondrocranium. Left, ventral view; right, dorsal view. The mandibular arches and the musclemesoderm of the hyal and branchial arches are shown. Dorsal roots of cranial nerves black, ventral roots omitted. Wavy lines diffuse blastema. Arteries striped. Contours of sucking discs and hypophysis broken lines. Dot-lining marks histologically and morphologically different elements bound together by blastema. Other indications as in Fig. 1. 100 ×.

anterior (m.r.a.) by means of a diffuse blastema. This muscle, in its turn, has a dorsal connection with the primordium of the m. rectus superior (m.r.s.) and a ventral one with that of the m. rectus inferior (m.r.i.) and the m. obliquus inferior (m.o.i.). In these muscle primordia myoblasts are differentiating. In fishes having a distinct premandibular cavity (Balfour 1874—78) the muscles mentioned have been shown to originate from the premandibular somite (see e.g.

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EDGEWORTH 1935). There is certainly no distinct head cavity to be seen in the mesenchymal cord in *Hepsetus* mentioned above, but its cells are rostrally quite loosely arranged inside the cord. This may indicate the presence of a reduced premandibular cavity. Because of that and of the fact that the premandibular eye muscles just mentioned at least in their posterior parts originate from the mesenchymal cord, it seems reasonable to suggest that the mesenchymal cord represents a *reduced premandibular somite*.

The *polar cartilages* (Figs. 2, 3, p.b.) consist each of a small blastema, situated between the primordium of the m. rectus externus (m.r.e.) dorsally and the carotis interna (Fig. 2, ci.i.) ventrally. Each polar cartilage is, because of the arrangement of the cells, detached from the trabecle anteriorly and the basiotic lamina posteriorly. The caudal connection is rather diffuse, which emphasizes the polar cartilages still more. Finally is to mention that the polar cartilages are laterally loosely connected with the processus basalis of the palatoquadrate (Fig. 1, pr.bas). This position medially to the mandibular arch is the only indication of their possible origin from a mandibular somite (cf. *Salmo,* HOLMGREN 1943).

Below the still undifferentiated otic vesicles the *otic cartilages* are differentiating. They initially appear in two parts, one anterior in the facial region and one posterior in the glossopharyngeal region (Fig. 2, ca.a., ca.p.). The anterior (part of the) otic cartilages have already reached the prochondral stage. Rostrally each anterior otic cartilage has a short postorbital process (Fig. 3, pr.po.).

Through the anterior basicapsular commissure (GAUPP 1900) (Fig. 2, a.bc.c.) and the basivestibular commissure (DE BEER 1937) (bv.c.), respectively, each anterior and posterior otic cartilage is connected with a blastemal cord below the medial border of the otic vesicles. This cord climbs on the lateral margin of the parachordal and extends back to the vagal region. Here it is continuous with the occipital part of the parachordal (pch.o.). If compared with the selachians (HOLMGREN 1940) and the lower teleostomes investigated by the present author (cf. below chapter IV) this cord should represent a *basiotic lamina*. As in the selachians, it is composed of different parts, ventrolaterally connected with the mesoderm of certain visceral arches. The anterior (facial) part (l.bas.a.) is connected with the mesoderm (the muscle rudiment) of the hyal arch (s.3.m.), and the middle or mesotic (glossopharyngeal) part (l.bas.m.) with the mesoderm of the first branchial arch (s.4.m.). Contrary to the conditions in the selachians, however, the mesoderm of the second branchial arch (s.5.m.) is connected with the basiotic lamina, too. This posterior part of the lamina is called the occipital basiotic lamina (l.bas.o.). It is situated in the vagal region and sends out a rostral process towards the posteroventral margin of the otic vesicle (cf. Fig. 5).

In the anterior basicapsular and the basivestibular commissures the blastema cells are stacked on each other. They gently merge into the otic cartilages but are medially distinctly discernable from the cord-like basiotic laminas with their more rounded and concentrically arranged cells.

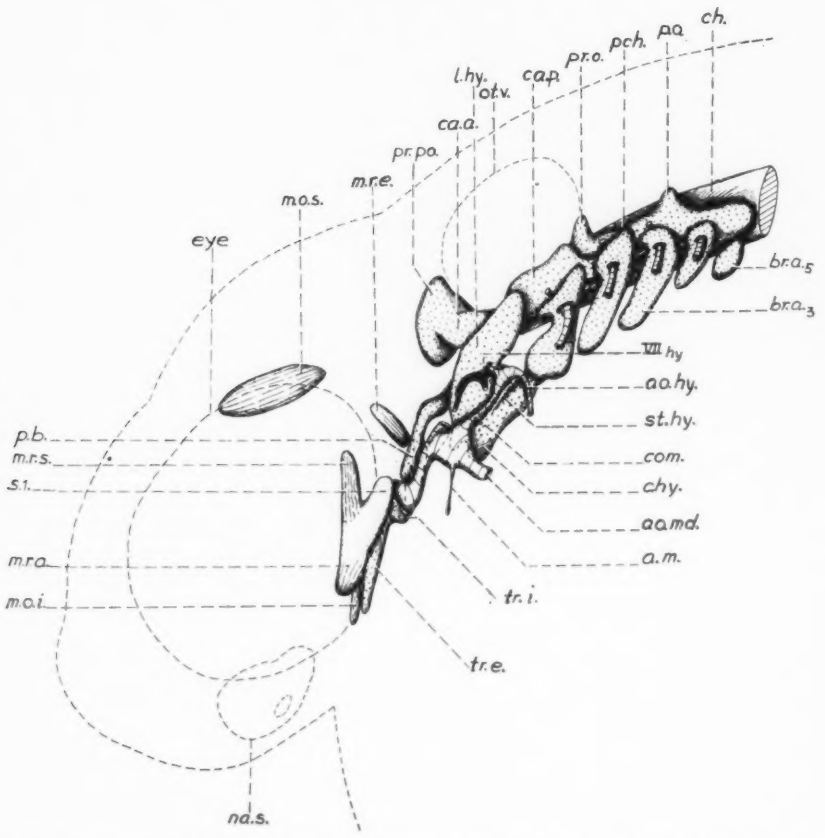

Fig. 3. *Hepsetus odoë*, 6.5 mm. Reconstruction of chondrocranium except mandibular arch, lateral view. Visceral nerves black and visceral aortas striped. Further indications as in Figs. 1—2. 100 ×.

Finally, the *parachordals* are situated in the cranial base, too (Fig. 2, pch.). They extend rostrocaudally between the basiotic laminas and the notochord and are both morphologically and histologically discernable from the basiotic laminas (cf. chapter IV). Like the laminas they consist of three parts, the anterior, the mesotic and the occipital parachordals (pch.a., pch.m., pch.o.). These are situated in the facial, the glossopharyngeal and the vagal regions, respectively, and are so far connected with the entire lateral surface of the notochord. The anterior parachordals (Holmgren 1940) consist of only sparsely nucleified blastema, whereas the two others are as densely nuclified as the basiotic laminas. However, all three have more irregularly arranged nuclei than the basiotic laminas.

The mesotic and occipital parachordals are each dorsolaterally connected with a neural arch (Goodrich 1930; de Beer 1937; and others), the preoccipital (Schreiner 1902) and the occipital arches (Allis 1897, pila occipitalia Stöhr

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1882, Occipitalspänge SCHREINER 1902) (pr.o., p.o.), respectively. The former arises caudally to the glossopharyngeal and the latter caudally to the vagal nerve root. Both arches extend on to the lateral side of the hindbrain. A preoccipital arch is earlier found also in *Lepisosteus* (SCHREINER 1902) and *Amia* (DE BEER 1924 a). Because it is situated dorsally to the mesotic basiotic lamina and later chondrifies as a ventromedial covering of the posterior semicircular canal in *Hepsetus*, the preoccipital arch may be homologous with the similarly situated selachian cartilage of the posterior semicircular canal (HOLMGREN 1940) or as HOLMGREN rather wants it to be called, "the cartilage of Källberg".

Visceral cranium

Contrary to many other teleosts described, *Hepsetus* has initially five separate skeletal branchial arches (Figs. 1, 3, br.a.). They extend back to the level of the first postotic myotomes. Together with the prootic (mandibular and hyal) arches they are of the usual gnathostomian type, i.e. located at the pharynx endoderm internally to the visceral nerves, muscles and vessels. All skeletal visceral arches are still undivided but in the prootic arches the principal elements can already be recognized owing to different density of their blastemas.

The morphology of the *mandibular arch* is very interesting since it resembles that of the other visceral arches in its essential respects (Fig. 4). The skeletal arch consists of a continuous blastema which dorsally is connected with a subepidermal, dorsolaterally situated blastema (pr.ot.e.). This is of the same histological type as that of the other visceral arches (l.hy., l.br.) (cf. p. 217). The connections with the skeletal mandibular arch lies anteriorly and posteriorly to the visceral nerve trunk, the visceral aorta (ao.md.) and the constrictor dorsalis muscle (co.md.d.). The visceral nerve trunk branches ventrally to the dorsolateral blastema into the bulky ramus (r.)mandibularis and r. maxillaris trigeminus ($V_{2.3}$). In the hyal ($VII_{m,h}$) and branchial arches (IX pt.) the visceral nerve trunk branches in a similar way. As to the constrictor muscle, finally, it should especially be noticed that it is formed of a continuous sheath in the mandibular arch, whereas in the other visceral arches it is formed as a dorsal and ventral portion.

The dorsomedial portion of the skeletal mandibular arch (pr.bas.) is polynuclear and loosely connected with the lateral part of the polar cartilage (p. 211). It remains so during the three first stages. When chondrifying, however, it separates from the polar cartilage. It is always situated medially to the nervus (n.) trigeminus and anteriorly to the r. palatinus facialis and the mandibular aorta (pseudobranchial artery). According to its position in relation to the cranium, nerves and vessels, it seems to represent the processus basalis in selachian embryos (HOLMGREN 1943), *Neoceratodus* (SEWERTZOFF 1902; and others), *Eusthenopteron* (JARVIK 1954), *Lepisosteus* (HAMMARBERG 1937), *Amia* (HOLMGREN 1943; by PEHRSON 1922 called proc. pediculus) and *Salmo* (DE BEER 1937).

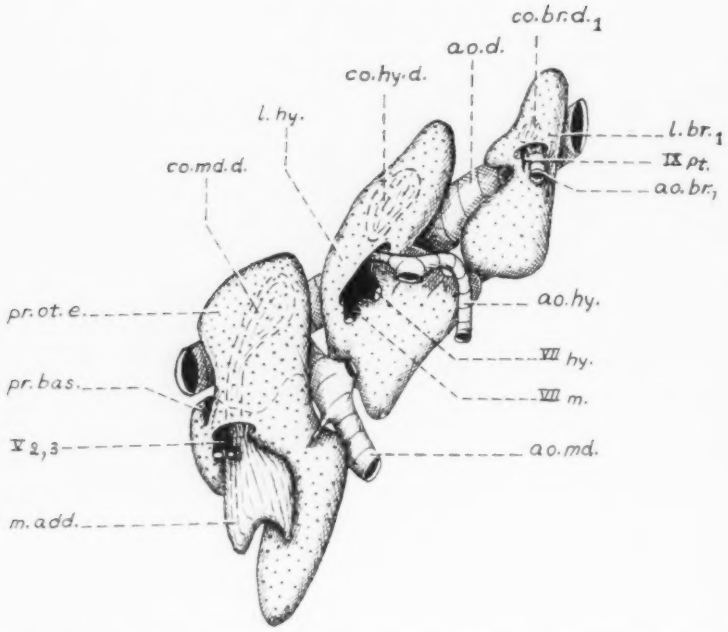

Fig. 4. *Hepsetus odoë*, 6.5 mm. Separate reconstruction to show the similar shape of mandibular, hyal and first branchial arches, and the comparable position in relation to visceral nerves, aortas, and muscles. Indications as in Figs. 1—2. 167 ×.

The dorsolateral blastema (Fig. 4, pr.ot.e.) is situated anteroventrally to the spiracle rudiment (Fig. 1, spir.). It rises from the dorsolateral margin of the pars quadrata (pa.qu.) and extends between the epidermis and the dorsal part of the m. constrictor dorsalis up to the postorbital process. It is limited by only sparsely nucleified mesenchyme from the neural cranium. The base is situated between the m. constrictor dorsalis and the m. adductor (Fig. 4, m.add.). HOLMGREN (1940, 1943) has, in selachians, described an extrapalatoquadrate blastema that forms independently below the epidermis and joins the posterior dorsolateral margin of the pars quadrata. This blastema is the rudiment of processus oticus externus (HOLMGREN). Since initially it has the same morphological relations to the spiracle, the m. constrictor dorsalis, the n. trigeminus and the postorbital process as the dorsolateral blastema in Hepsetus, the two elements seem to be homologous.

The pars quadrata (Fig. 1, pa.qu.) is thick and densely nucleified. It is turning prochondral. That circumstance as well as the different arrangement of its cells make it possible to distinguish it from the lower jaw (md.). This is laterally covered by a blastemal sheath, from which later dentary and angular will differentiate.

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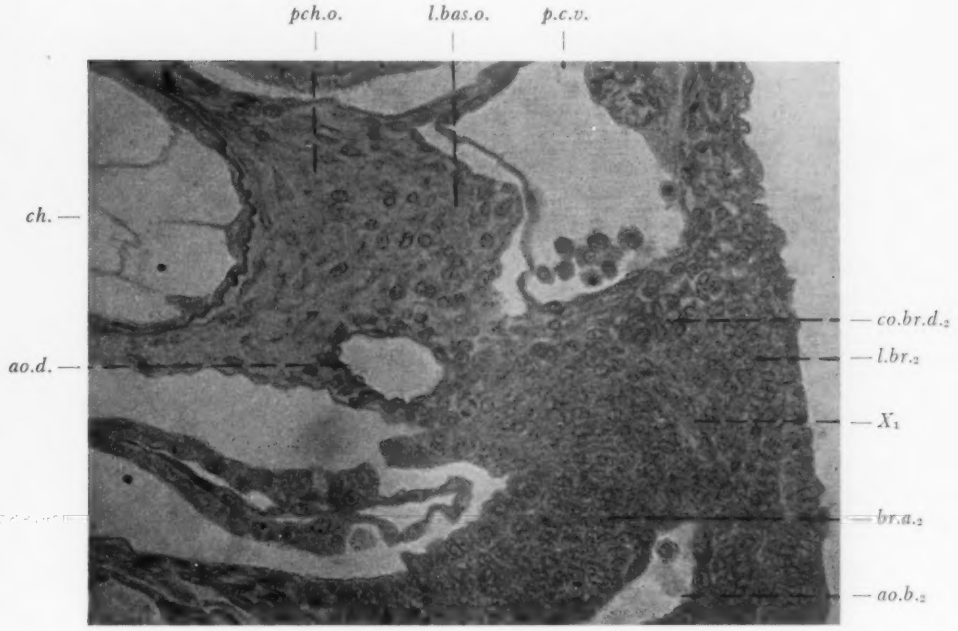

Fig. 5. *Hepsetus odoë*, 6.5 mm. Transverse section through right part of skull, immediately posterior to the otic vesicles and on a level with the second branchial arches. 550 ×.

Rostrally the pars quadrata is connected with the processus pterygoideus (pr. pt.); this is still a very short blastemal bar situated along the rostral part of the pharynx endoderm. The pterygoid process rostrally merges into a part of the skeletal mandibular arch (md.a.), in which no differentiation into chondro- and osteoblasts has yet appeared. This part medially joins the rostrolateral end of the trabecle (Fig. 2) and then proceeds forwards below the eye and up to the nasal sac. Here, in front of the hypophysis (hyp.), it is joined to the mandibular arch of the other side, forming a bridge of scattered mesenchymal cells, which in this paper is called the *anterior mesenchyme* (a.mes.). The origin of this mesenchyme has not been histologically traced. However, if considered (1) that it is continuous with the rostral end of the mandibular arches and the trabecles, (2) that it differentiates (with the beginning in stage III) into chondroblasts forming the rostral parts of the trabecles (and the ethmoidal plate) and (3) that these portions, according to comparative and experimental embryological reasons, probably are of ectomesenchymal origin (cf. p. 295), it seems reasonable to suggest that also the anterior mesenchyme has the same source.

HOLMGREN (1943) has described a "frontal mesenchyme" of exactly the same position in *Salmo salar*. He considered it, however, to be primarily of entodermal origin and corresponding to the "präorales Entoderm" of KUPPFER (1893) and

perhaps also to the "premandibular mass" of DOHRN (1902) in selachians. Those comparisons shall not be considered here but it should be pointed out that HOLMGREN later in his account admits that the frontal mesenchyme "becomes so mingled with ecto-mesoderm that a good outline could not be drawn ... There thus exists a possibility that ectomesodermal cells could take part in the formation of the most anterior portion of the trabecular system". In the light of the results of experimental embryology this possibility is very strong. Then, it is also very probably that the original entodermal frontal mesenchyme in *Salmo* is, in this region, substituted by ectomesodermal mesenchyme (ectomesenchyme, cf. p. 208, note 1), homologous with the anterior mesenchyme in *Hepsetus*. However, further investigations are needed in this matter.

Also the *hyal arch* is comparatively large and runs from the posterior wall of the spiracular canal vestige (Fig. 1, spir.). It is very interesting to note the principal resemblance between the hyal and the mandibular arches in early stages (cf. p. 213), the only fundamental difference being that the latter has a pterygoid process. However, as will be discussed in chapter V, it is possible that this process does not belong to the mandibular arch but represents an epal element of a premandibular arch (p. 342).

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Then several noticeable similarities with the mandibular arch are to be noted: (1) The dorsal portion, the hyomandibula (hy.), is divided into a dorsomedial and a dorsolateral portion, having different histological appearance (cf. p. 217). (2) The border between them is accentuated by the visceral nerve, truncus (tr.) hyomandibularis facialis (Fig. 4, tr.hy.), the visceral aorta (ao.hy.) and the m. constrictor hyalis dorsalis (co.hy.d.). (3) The dorsomedial portion is directed towards the cranial base. (4) The dorsolateral portion, here called the laterohyal (l.hy.), points towards the lateral part of the neurocranium.

In the details, however, there are differences between the hyal arch and the mandibular arch. The distance between the exits of nerve and aorta is considerably shorter in the hyal arch, approaching the branchial arch type (Fig. 4). Further, the hyal arch has a short and slender but quite distinctly visible process, the symplectic (Fig. 1, sy.). This points rostrad and is situated medially to the commissural artery (Fig. 3, com.) at the point where this joins the mandibular aorta. Finally, another element peculiar to the hyal arch can be seen, viz. a stylohyal (interhyal) (st.hy.). It has just begun to differentiate from the middle of the posteromedial part of the continuous skeletal hyal arch. The stylohyal is comparatively thick and has a lateral, subepidermal part continuous with the more densely nucleified dorsolateral blastema, the laterohyal. Anteriorly to the stylohyal the ceratohyal (c.hy.) is continuous with the base of the symplectic (Fig. 1).

Finally, a certain point of interest may be emphasized as to the hyal arch. Fig. 4 shows that *Hepsetus* has a complete hyomandibular aorta joining the dorsal aorta (ao.d.). It is true that the dorsal portion, the efferent hyal artery (Fig. 9,

eff.hy.) or dorsal hyal aorta (KRYZANOWSKY 1934), is thin. However as it has been observed in four specimens and is still found even at the next stage (7.3 mm), it cannot be an artefact. In his close study of the pseudobranchs in fishes KRYZANOWSKY (1934) has shown that this type of hyomandibular aorta exists only in selachians (adult), and in embryonic stages of *Acipenser, Amia, Lepisosteus, Esox* and *Carassius*. This type is said to be primitive.

There are, in this early stage, five completely separate skeletal *branchial arches* (Figs. 1, 3, 4). They are each represented by a somewhat curved bar of a dense blastema, situated in close contact with the lining epithelium of the branchial cavity. The cell nuclei are arranged with their length axis perpendicularly to the pharynx endoderm and to the course of the branchial arch (Fig. 5).

As is seen from Fig. 4 and as was also mentioned earlier (p. 213) there is a principal similarity between the branchial arches and the prootic arches. Anteriorly and posteriorly to the visceral nerve, the visceral aorta and the m. constrictor dorsalis the dorsal part of each skeletal branchial arch—except the fifth—is connected with a blastema of the same position and type as the dorsolateral blastema of the prootic arches, i.e. it is situated below the epidermis, laterally to the constrictor dorsalis muscle and extending towards the lateral part of the neurocranium; the nuclei are irregularly arranged, sligthly more stainable in Azan and of smaller size than those of the inner (skeletal) arch. Finally, the dorsolateral blastemas are ventrally continuous with a thinner subepidermal sheath, laterally enclosing the visceral aorta and the visceral nerve. In the prootic arches this sheath may chondrify in connection with the skeletal arch (part of the stylohyal, the ventral portion of the processus oticus externus), but most of it vanishes or becomes connective tissue; in the branchial arches, however, it differentiates into gill rays (GOODRICH 1909; filamentary cartilages DANFORTH 1912; gill filaments WOODWARD 1895; Kiemenstäbchen GAUPP 1904).

Stage II, 7.3 mm (Figs. 6—12).

The external morphology is still dominated by the big yolk sac, the eyes (Fig. 6) and the sucking discs (s.d.) and the inner morphology by the notochord, the ear vesicles and the nasal sacs. The cranium is a little more differentiated but still blastemal in essential parts.

Neural cranium

The caudal part of the premandibular somites (Fig. 7 A, s.l.) are still histologically and morphologically discernable. The premandibular eye-muscle rudiments are sligthly more differentiated.

The *trabecles* have grown backwards and joined the polar cartilages (p.b.) even laterally (Fig. 8, tr.e.) to the carotis interna (c.i.). As they are still connected with the polar cartilages medially to the carotid and because the polar cartilages are situated dorsally to the carotid, there is formed a carotid foramen

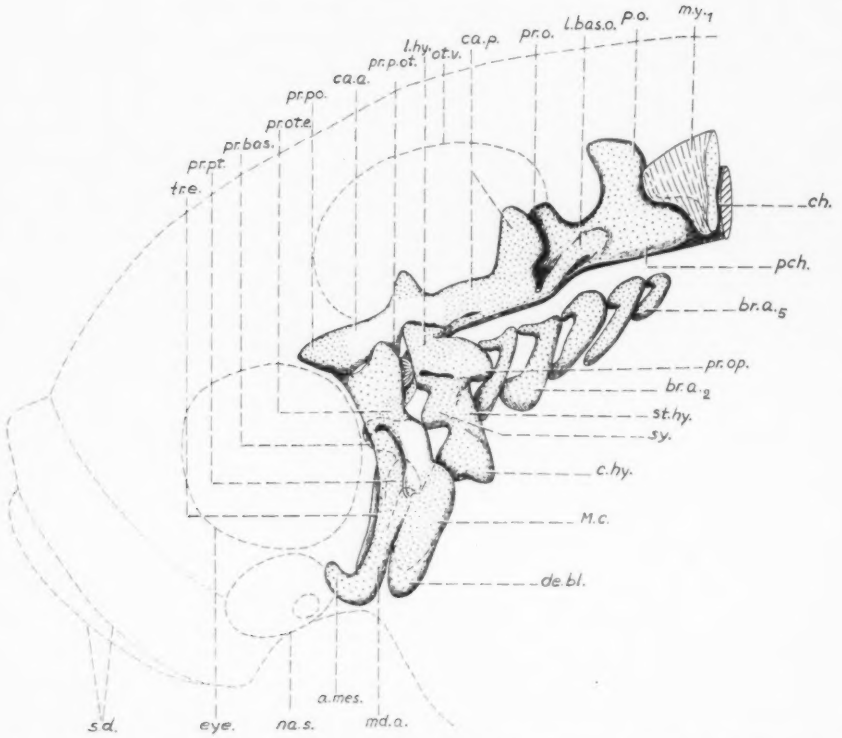

Fig. 6. *Hepsetus odoë*, 7.3 mm. Reconstruction of chondrocranium, lateral view. Indications as in Figs. 1—2. 92 ×.

(fo.c.) between the caudal end of the trabecles and the polar cartilages. The carotid runs through this foramen. According to the position this foramen seems to be homologous with the carotid foramen in *Acipenser* (SEWERTZOFF 1923, cf. p. 298). The difference is that only the lateral margin will chondrify in *Hepsetus* (p. 298).

The *polar cartilages* are still loosely connected with the anterior basiotic laminas (l.bas.a.). Dorsally to the posterior portion of the polar cartilages a blastemal process (Fig. 7, pr.p.ot.) is growing ventrad from the lateral side of the anterior otic cartilages immediately in front of the hyomandibulae. It is situated between the secondary vein and the facial ganglion medially and the spiracular canal vestige laterally. A similarly situated process has been described by other authors, e.g. by PEHRSON (1922) in *Amia* and by DE BEER (1937) in *Salmo fario*. It is usually called processus prooticus; later it takes part in the forming of the dorsal part of the lateral commissure (cf. p. 226).

By means of a blastemal bridge the prochondral anterior *otic cartilages* (Figs. 6, 8, ca.a.) have laterally joined the blastemal posterior otic cartilages (ca.p.).

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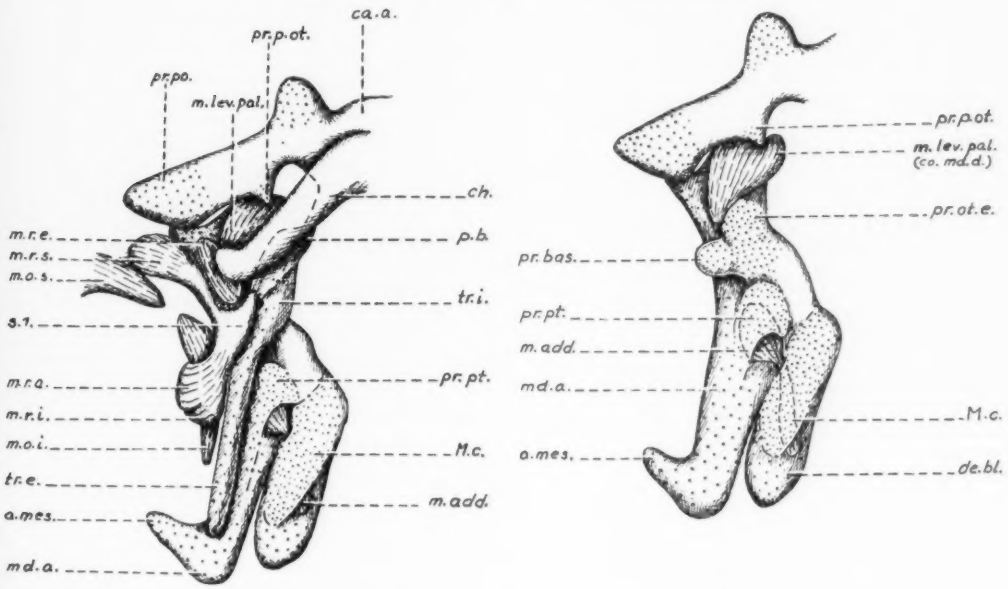

Fig. 7. *Hepsetus odoë*, 7.3 mm. A, reconstruction of anterior part of chondrocranium, medial view. 92 ×. B, reconstruction of only mandibular arch and anterior part of otic cartilage, medial view. 92 ×. Indications as in Figs. 1—2.

Then a foramen has been formed below each otic vesicle (ot.v.), the fenestra basicranialis anterior (Fig. 8, fe.b.$_1$). Almost the entire opening is filled with the large secondary vein. Dorsally to this fenestra the prospective utriculus is beginning to differentiate as a medial thickening of the otic vesicle. The acustic ganglion is situated close to the medial border of the fenestra dorsally to the anterior basiotic lamina. The anterior basicapsular commissures (Figs. 8, 9, a.bc.c) have become prochondral and hence a comparatively well differentiated bar has been formed below the frontal part of each otic vesicle. A similar morphology of the region is described also in early stages of other teleosts, e.g. *Leuciscus rutilus* (Hubendick 1943).

The *parachordals* and *basiotic laminas* are comparatively unchanged. The anterior parachordals (pch.a.), however, are now blastemal and more densely nucleified. Further, the rostral process of the occipital basiotic laminas (Fig. 8, l.bas.o.) have fused with the mesotic laminas (l.bas.m.), together forming a ventral covering for the differentiating posterior part of the otic vesicles (the prospective lagena). The mesoderm of the visceral arches is differentiating into muscle tissue and has simultaneously lost the connection with the laminas. The occipital arches (Figs. 6, 8, p.o.) have grown dorsorostrad and approached the otic vesicles, whereas the preoccipital arches (pr.o.) are unchanged.

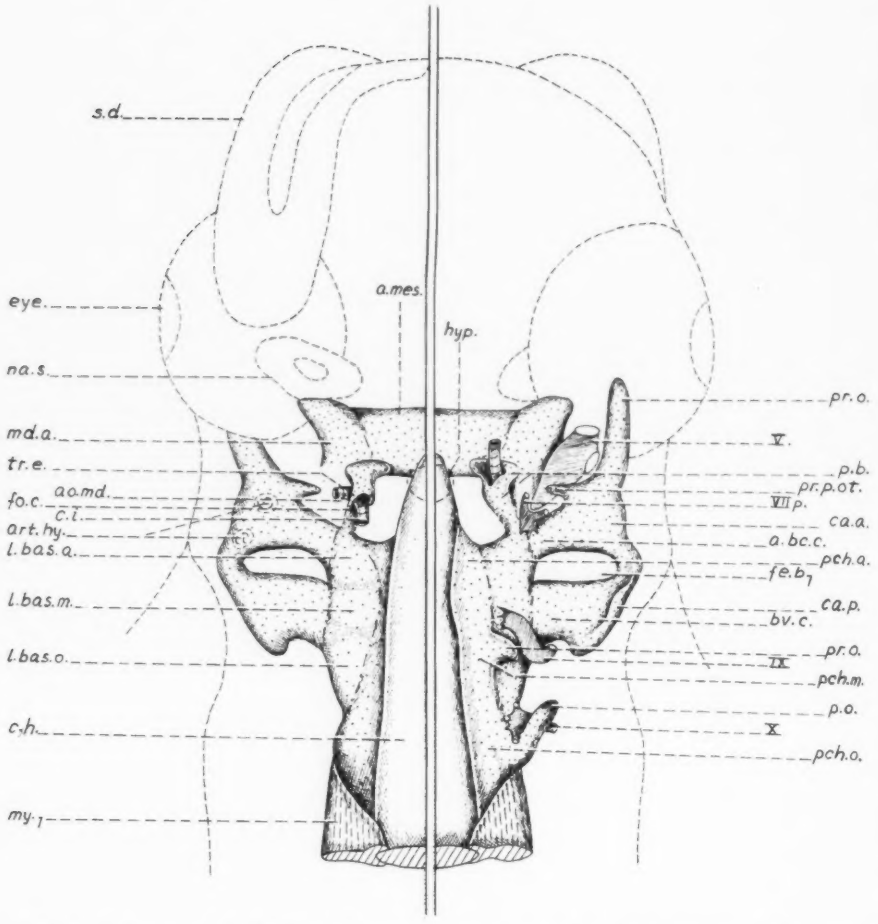

Fig. 8. *Hepsetus odoë*, 7.3 mm. Reconstruction of neural chondrocranium. Left, ventral view; right, dorsal view. The relation to dorsal roots of the cranial nerves is shown. Indications as in Figs. 1—2. 92 ×.

Visceral cranium

Simultaneously with deepening of the head fold and the rising of the head from the yolk sac, the branchial arches join ventrally and connect with each other dorsally (Figs. 6, 10). Each of the skeletal visceral arches is still undivided, consisting of a somewhat curved bar of dense blastema, lying in close contact with the lining epithelium of the pharynx. Gill septa have started to form in the anterior branchial arches.

The *mandibular arch* has in its posterior part become dorsoventrally elongated (Figs. 6, 7) and the m.constrictor is now completely divided into a dorsal portion,

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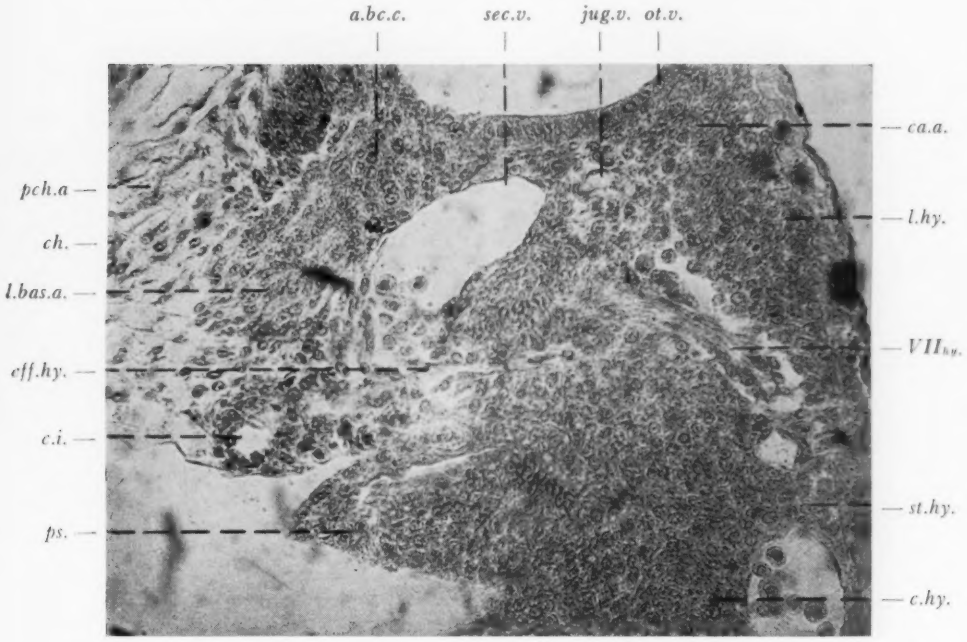

Fig. 9. *Hepsetus odoë*, 7.3 mm. Transverse section through right part of skull, on a level with the hyal arch. The section shows the position of anterior parachordal and basiotic lamina, laterohyal and vestigial efferent hyal artery. 320 ×.

the m.levator arcus palatinus (m.lev.pal.) and a ventral part, m.adductor (m. add.). Dorsally the processus oticus externus (pr.ot.e.) now lies in direct contact with the prootic process of the anterior otic cartilage (cf. Acanthodians and old shark types such as *Cladoselache* etc., HOLMGREN 1943).

Rostromedially the pars quadrata is fused with the pterygoid process (Figs. 6, 7, pr.pt.). As before this process makes an indentation in the pharynx wall. Rostrally it merges into the undifferentiated part of the mandibular arch, which has about the same extention as before (md.a.).

In the *hyal arch* skeleton the different elements are now forming (Fig. 6). They are, however, still blastemally connected with each other. Dorsally the tr.hyomandibularis, the efferent hyomandibular artery (Figs. 9, 11, eff.hy.) and the m.constrictor hyalis dorsalis show as before the limit of the two histologically different dorsal portions of the hyomandibula. The laterohyal (l.hy.) is histologically unchanged but has dorsally reached the anterior otic cartilage and posteriorly developed an opercular process (Fig. 6, pr.op.). The symplectic and the stylohyal are still rather unchanged.

The *branchial arches* have met dorsally. Their middle parts (ceratobranchialia) have differentiated into prochondrium; the rest is still blastemal.

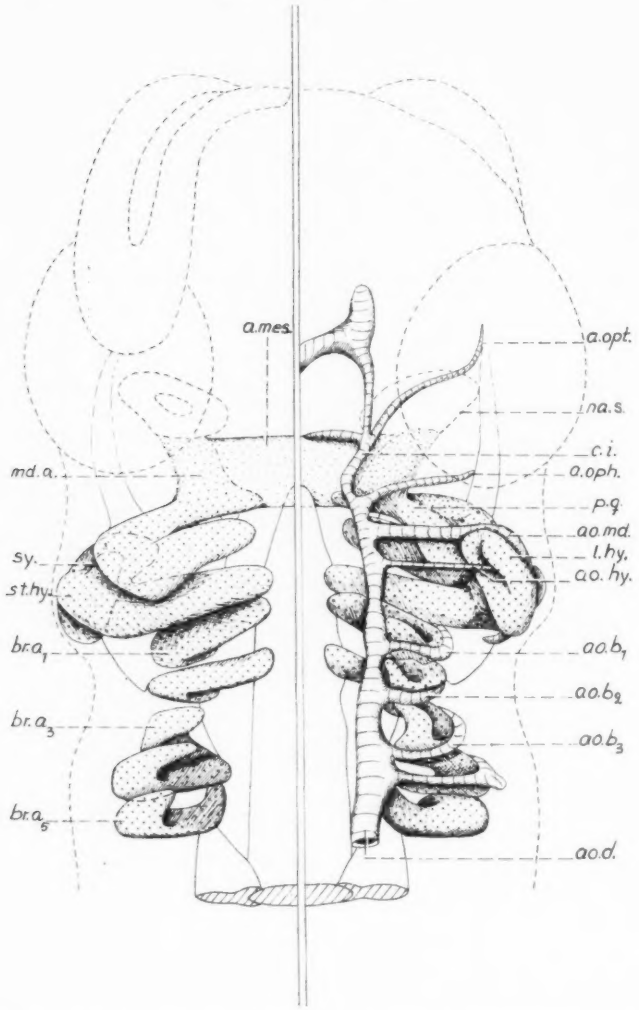

Fig. 10. *Hepsetus odoë*, 7.3 mm. Reconstruction of visceral chondrocranium. Left, ventral view; right, dorsal view. The position of the visceral arches in relation to visceral arteries are shown to the right. Neural cranium is indicated by thin continuous lines. Other indications as in Figs. 1—2. 92 ×.

The dorsal part of the first two arches have started to develop a rostromedial process, laterally to the dorsal aorta (Figs. 10, 11, ao.d.). Later the anterior part of each process will chondrify independently, forming an infrapharyngobranchial element, whereas the posterior part forms an epibranchial one. Further, from the posterior portion of the prospective epibranchial element, a process arises posteromedially to the visceral nerve and aorta. This process later chondrifies in con-

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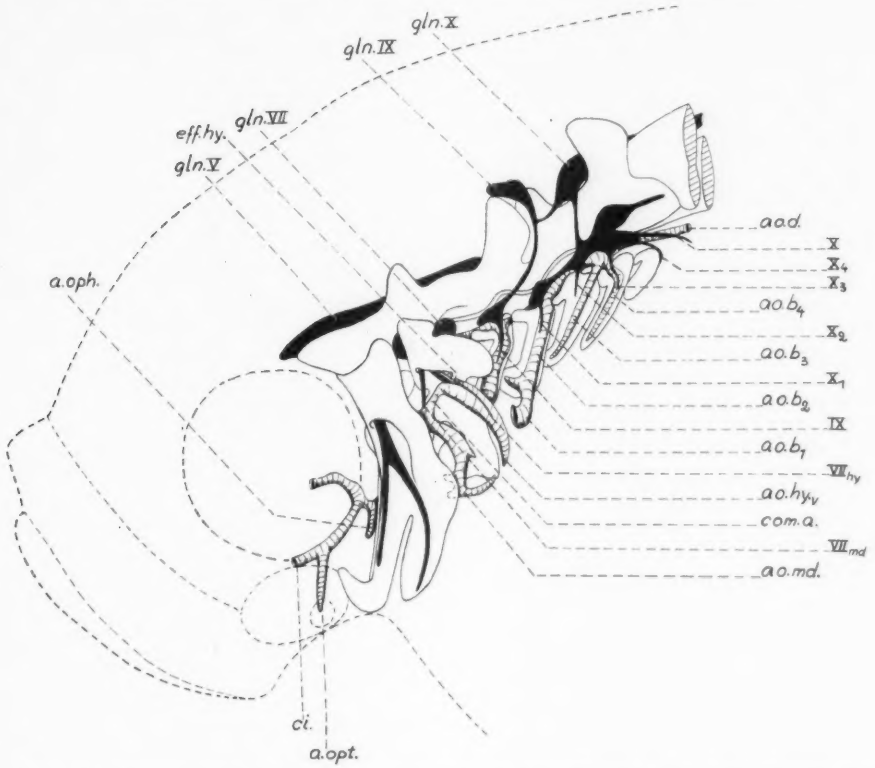

Fig. 11. *Hepsetus odoë*, 7.3 mm. Reconstruction of chondrocranium to show position and course of visceral nerves (black) and visceral arteries (striped). Lateral view. 92 ×.

nection with the epibranchial. If compared with *Acipenser* (HOLMGREN 1943), *Eusthenopteron* (JARVIK 1954) and other fishes (cf. p. 305), this process is a suprapharyngobranchial. In *Acipenser* it chondrifies separately; this difference seems, however, not to be of hindrance to the homologization (p. 305).

Laterally to the suprapharyngobranchial process the dorsolateral blastema is about to differentiate into dorsal gill rays.

Stage III, 8 mm A (Figs. 12—14).

The head fold is still deepening and the head is continuing to rise from the yolk sac. The contour of the head is, therefore, getting more fishlike (Fig. 12). The adhesive organ (s.d.) is growing in size and dominates the anterior external morphology. The cranium is still mainly blastemal but in certain portions it is also chondrifying.

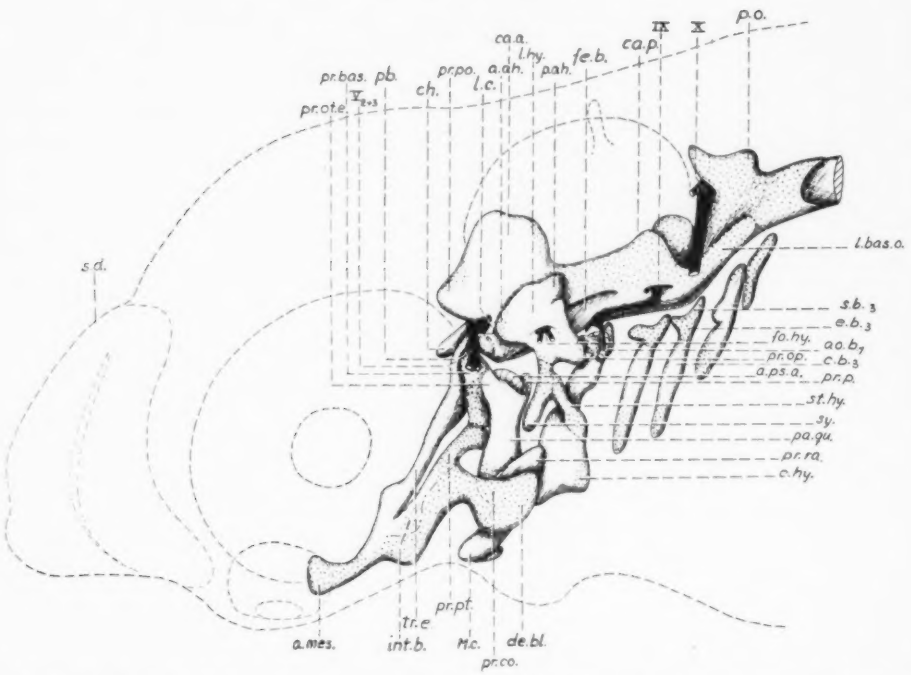

Fig. 12. *Hepsetus odoë*, 8 mm A. Reconstruction of chondrocranium, lateral view. The relation to visceral nerves and arteries is indicated. Indications as in Figs. 1—2. 83 ×.

Neural cranium

The *trabecles* are approaching each other frontally (Fig. 13, tr.e.). However they have not yet joined but are still connected by means of the anterior mesenchyme (a.mes.). Caudally the trabecles are narrower and turning prochondral. The premandibular somites are not possible to discern any more.

On the level of the hypophysis (hyp.) the trabecles are now fused with the likewise chondrifying polar cartilages without any demarcation (p.b.). The lateral of the two connections on each side of the carotid (c.i.) has become dominating; the medial connection (tr.i.) is left but is still blastemal and has thinned out.

The *polar cartilages* are caudally protochondrally connected with the anterior basiotic laminas (l.bas.a.). There is still, however, a morphological demarcation left between them (Figs. 12—13). To the earlier demarcation line between the *basiotic laminas* and the anterior basicapsular and the basivestibular commissures (Fig. 13, a.bc.c., bv.c.), respectively, a demarcation also between the anterior and the mesotic basiotic laminas has in this stage, been added. Their cells have arranged differently during the chondrification process (cf. Fig. 19). The earlier morphological difference between them has then become strengthened by a histological one. This demarcation will remain up to the adult stage.

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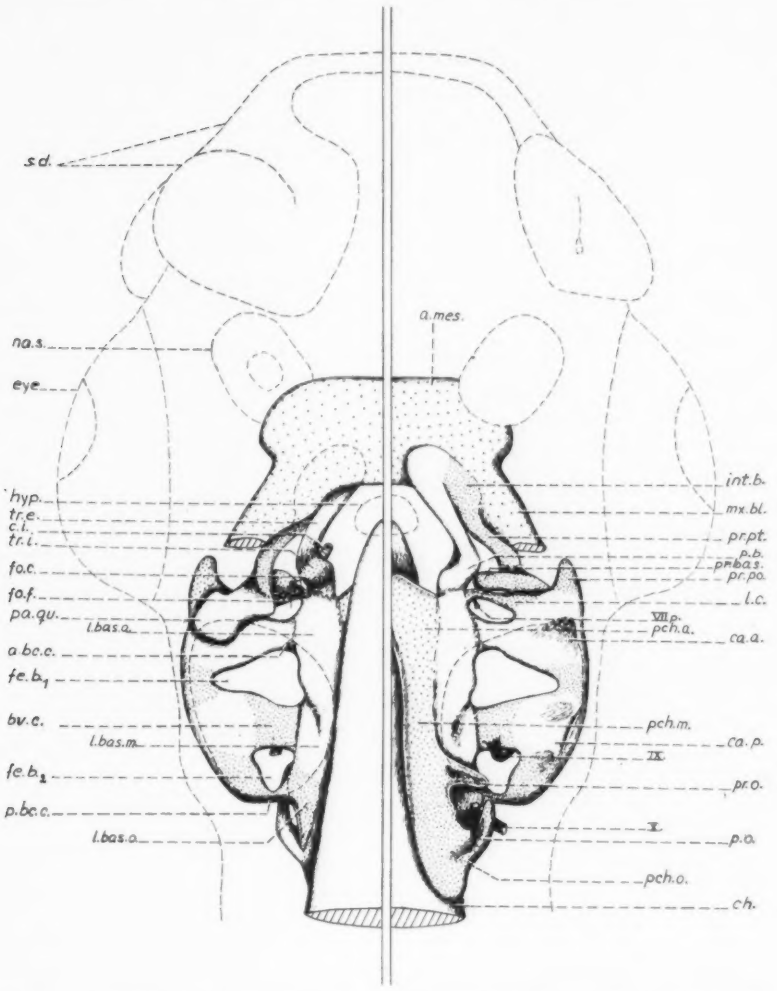

Fig. 13. *Hepsetus odoë*, 8 mm A. Reconstruction of neural chondrocranium and palatoquadrates. Left, ventral view; right, dorsal view. Dorsal nerve roots indicated on the right side. Sparcely dotted area mesenchyme, densely dotted area blastema. Dot-lining indicates histologically and morphologically different elements bound together by blastema or, after chondrification, by a common perichondral membrane. Other indications as in Figs. 1—2. 83 ×.

The *parachordals* are morphologically slightly changed. The blastemal anterior parachordals (pch.a.) are now situated only on the dorsolateral side of the notochord (ch.) and have fused with their mates above the notochord; in this way a *basal plate* is formed. The strong growth of the occipital arches (Figs. 12, 13,

p.o.) that began in the former stage has continued. They have now reached the otic vesicles (ot.v.).

The anterior (ca.a.) and the posterior *otic cartilages* (ca.p.) are turning protochondral but are still only blastemally connected with each other. In the vagal region a blastemal posterior basicapsular commissure (p.bc.c.) was, at the former stage, beginning to develop from the posterior otic cartilages. It has now reached the occipital basiotic lamina on each side. Thus a posterior basicranial fenestra (fe.b.$_2$) has been established, through which the glossopharyngeal nerve (IX) runs.

Another blastemal process developing in the former stage was the prootic process (p. 218; Figs. 6—8, pr.p.ot.). It has now grown ventromediad and reached the rostrolateral end of the anterior basiotic lamina. A thin and narrow bridge (Figs. 12, 13, l.c.) is then formed between the exits of the n.trigeminus (anteriorly), the r.palatinus (VIIp) and tr.hyomandibularis facialis (posteriorly) and laterally to the secondary vein, the orbital artery and the facial ganglion. According to the prevailing opinion this lateral wall of the trigemino-facialis chamber is the *lateral commissure* (DE BEER 1927, 1937) and the foramen thus formed posterior to it, the foramen facialis (fo.f.).

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Visceral cranium

The differentiation of the visceral arch skeleton has principally been localized to the prootic arches.

The *mandibular arch* is now taking form more definitely. The original division of the pars quadrata into a dorsomedial (proc. basalis) and a dorsolateral (proc. oticus externus) part can, however, still be seen (Fig. 12, pr.bas; pr.ot.e.). The processus oticus externus has grown slightly in the ventral part and is here differentiated into prochondrium; the remaining portion extends in the form of a thin blastemal sheath outside the m.constrictor mandibularis dorsalis (levator arcus palatinus). This sheath later vanishes altogether and can not be traced at the next stage. The basal process, on the contrary, will remain entirely. The ventral portion has become prochondral, whereas the dorsal portion is still blastemal and only loosely connected with the polar cartilage.

The essential part of the pars quadrata is chondrifying. Caudally is, however, still a blastemal portion left medially to the mandibular aorta. Part of it will later chondrify as a short process of the posterior margin of the pars quadrata, secondarily excavated to give place for the mandibular aorta (pseudobranchial artery) (Figs. 12, 14, pr.p.). It is situated medially to the dorsal portion of the mandibular aorta (the afferent pseudobranchial artery). This process is not described in teleosts earlier. In this paper it has been called the *processus posterior*.

The pars quadrata (pa.qu.) has now become almost completely separated from the meckelian cartilage (M. c.). In the prospective jaw-joint there is, however, still a rostral connection, joining the processus coronoideus of the mandibula (Fig. 14, pr.co.). These two processes were formed already at the former stage although

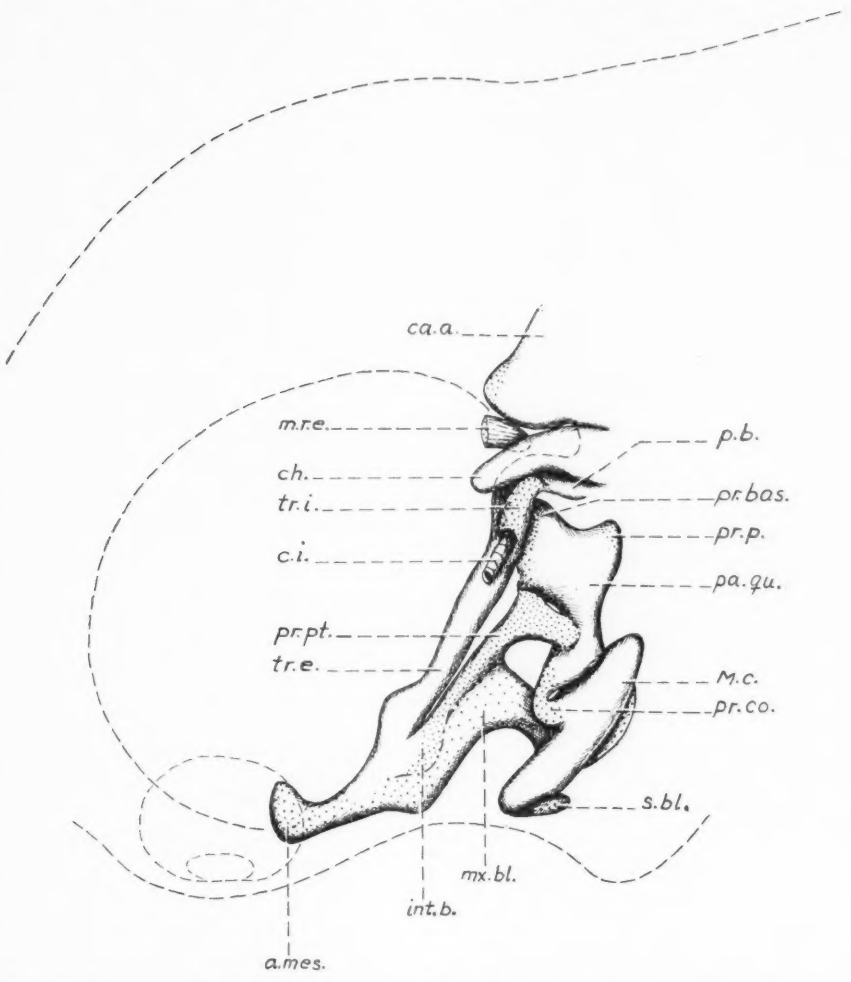

Fig. 14. *Hepsetus odoë*, 8 mm A. Reconstruction of anterior part of chondrocranium, medial view. Indications as in Figs. 1—2, 13. 133 ×.

still rather diffuse (Fig. 6). As will be shown later there is a striking resemblance between this frontal connection in the jaw-joint and the one between the symplectic and the stylohyal in young *Lepisosteus* embryos (p. 340).

Also the meckelian cartilage is chondrifying. Caudally to the future jaw-joint, there is, however, still a blastemal portion, processus retroarticularis (Fig. 12, pr.ra.). This points towards and is even blastemally connected with the ceratohyal (c.hy.). This connection will later form a ligament. No perichondral ossification of the meckelian cartilage has yet begun, but a lateral blastema indicates the place of the prospective dentary (de.bl.). It runs parallell with the meckelian

cartilage back to the corner of the mouth. Then it turns anterolaterad as a maxillary blastema (mx.bl.), and joins the ventral margin of the pterygoid process (pr.pt.). Further forward it joins the anterior blastema (a.mes.).

The processus pterygoideus is still blastemal and is as usual situated as an indentation of the anterior part of the pharynx. Since the pars quadrata is turning protochondral and its cells are stacked on top of each other, whereas the pterygoid process is only blastemal and has concentrically arranged nuclei, the pterygoid process is now better discerned from the pars quadrata (Fig. 14). Rostrally the pterygoid process can now be followed up to the connection with the trabecle. It consists of a polynuclear blastema of slightly larger and weaker stainable nuclei than those of the maxillary blastema. A thickening of the pterygoid process is found at the connection with the trabecle. This part corresponds to the *intermediating body* (int.b.), established and illustrated by HOLMGREN (1943) in early stages of *Acipenser, Amia* and *Salmo* (p. 343). Further, the pterygoid process is along the entire length connected with the trabecle by means of a blastemal sheath, posteriorly thinning out and vanishing. This sheath has nothing to do with the forming of the lateral commissure (cf. *Salmo;* HOLMGREN 1943) but is only connected with it. The basal process, on the other hand is formed in the posterior part of this sheath (cf. sharks, acipenserids and holosts; HOLMGREN 1943).

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The *hyal arch* has grown dorsoventrad and is chondrifying. The laterohyal (Fig. 12, l.hy.) has become elongated anteroposteriorly and has formed an anterior (a.ah.) and a posterior articular head (p.ah.). On the border between the laterohyal and the rest of the hyomandibula the foramen hyomandibularis (fo.hy.) for the tr. hyomandibularis VII has been narrowed down and the efferent hyal artery, which at the former stages was passing through the foramen, has now obliterated altogether. The symplectic (sy.) has grown anteroventrad and is chondrifying independently of the hyomandibula and the stylohyal (st.hy.). The same applies to the stylohyal.

The *branchial arches* are rather unchanged in their morphology and no division of the arches is yet visible. Histologically, however, they have changed as chondrification has begun in the ceratobranchials (c.b.). The remaining part of the arches, including the suprapharyngeal processes (s.b.), is still essentially blastemal.

Stage IV, 8 mm B (Figs. 15—22).

The yolk sac has been resorbed still more and the head fold continued to deepen (Fig. 15). The visceral arches then have separated from the yolk sac, and are free to fuse ventrally. Because the framing of the eye has begun also dorsally, the adult cranium is now essentially outlined (Figs. 15, 16). The adhesive organ (s.d.) is, however, still very large and dominates the snout. Most of the earlier formed cranial elements are chondrifying.

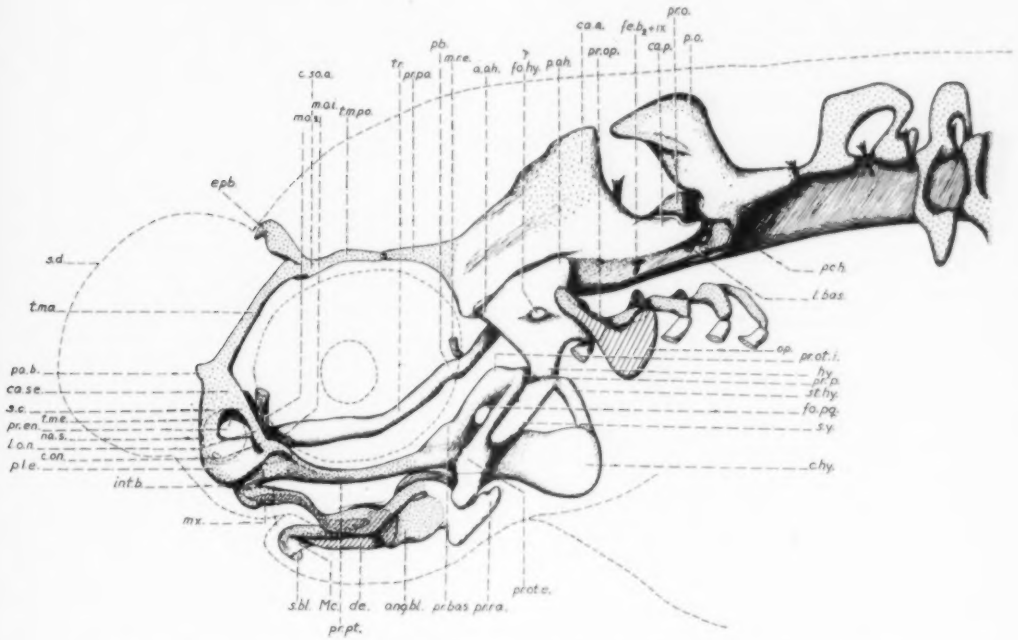

Fig. 15. *Hepsetus odoë*, 8 mm B. Reconstruction of chondrocranium, lateral view. Indications as in Figs. 1—2, 13. 55 ×.

Neural cranium

As almost the entire head is now released from the yolk sac, the anterior part of the head is turning upwards and in the polar cartilage region (p.b.) the earlier angel between the otic and the orbitotemporal regions is straightening out (Fig. 15).

Another striking fact is that the cranium has now turned tropibasic (Fig. 16), as the *trabecles* have connected back to the optic chiasma, forming a trabecula communis (tr.com.). In this way a fenestra basicranialis (f.b.) has been established. Rostrally to the trabecula communis the anterior mesenchyme (a.mes.) is chondrifying, forming a trabecular commissure (tr.c.). Such an element is also seen in *Amia* (Pehrson 1922), *Lepisosteus* (Veit 1911, Hammarberg 1937) and in *Polypterus* (Moy-Thomas 1933); it is, however, broader there. It is also described in *Salmo* (Gaupp 1905, Parker 1873, de Beer 1927) although small as in *Hepsetus*. In other teleosts it is often missing.

Anterior to the trabecular commissure other parts of the anterior mesenchyme have differentiated into a protochondral planum ethmoidale (Swinnerton 1902) (Figs. 15—17 A pl.e.). Other terms are "subnasal lamina" (Parker 1873),

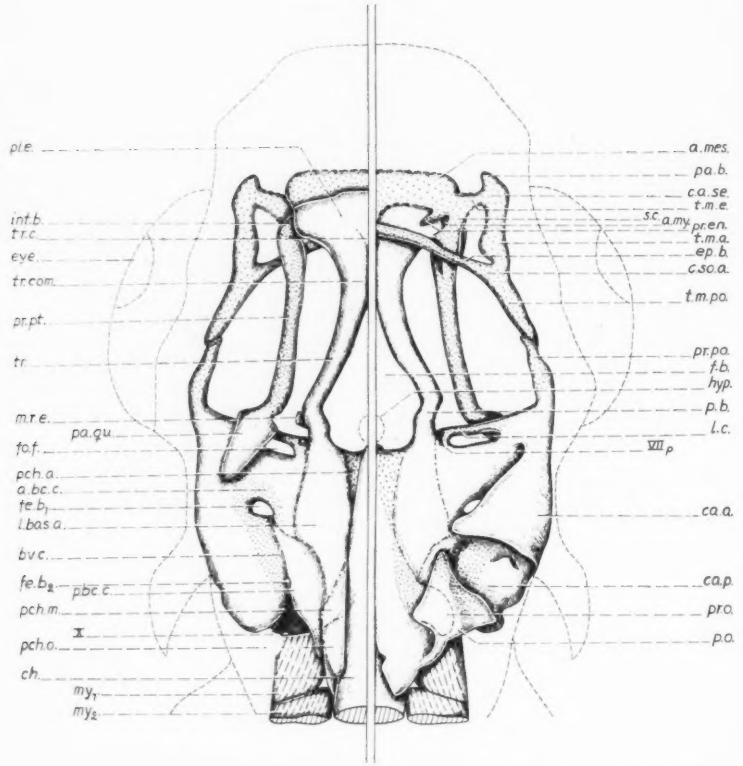

Fig. 16. *Hepsetus odoë*, 8 mm B. Reconstruction of neural chondrocranium and palatoquadrates. Left, ventral view; right, dorsal view. Indications as in Figs. 1—2, 13. 55 ×.

"rostral plate" (Mc Murrish 1883) and "planum orbitonasale" (Veit 1902). Much of the anterior mesenchyme is, however, still left under and anterior to the ethmoidal plate. This portion corresponds to the palatoquadrate commissure (Fig. 17 A, com.pq.) in *Salmo* and other teleosts (Holmgren 1943); it will form chondrocytes for the ethmoidal plate but also osteoblasts for the premaxillary, the vomer and the dermopalatine (cf. Fig. 17 B).

The trabecular connection with the polar cartilages medially to the carotis interna has vanished. Accordingly the carotid foramen present in early embryonic stages does not remain at the adult stage as in *Acipenser* (Sewertzoff 1922).

The *polar cartilages* (Figs. 15, 16, p.b.) are now without any demarcation fused both to the trabecles and to the basiotic laminas. Their position can, however, be traced morphologically as a short horizontal portion (seen from the lateral side) between the posterior end of the external rectus muscle (Fig. 18, m.r.e.) and the junction between the efferent pseudobranchial artery (e.ps.a.) and the carotis interna (c.i.). Also in the horizontal plane the polar cartilages can be traced as

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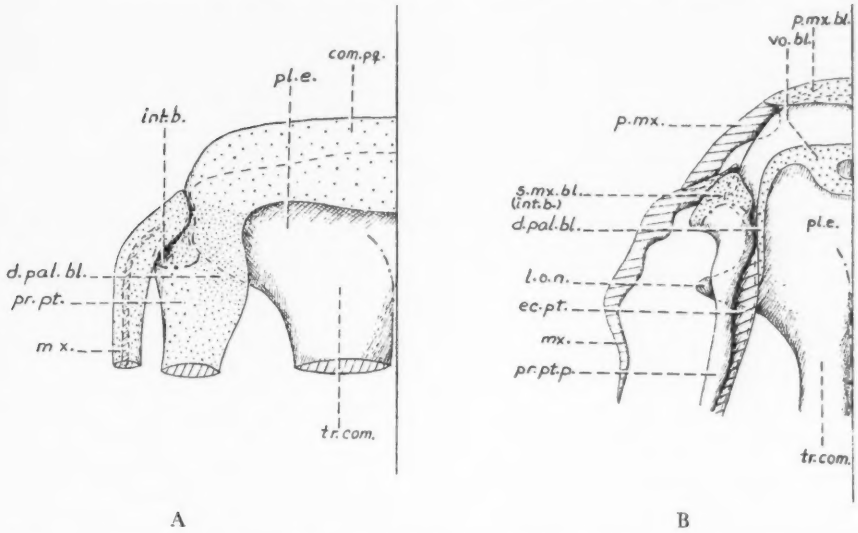

A B

Fig. 17. *Hepsetus odoë*. Reconstruction of ethmoidal region, ventral view. A, 8 mm B. 128 ×. B, 12.6 mm. Bone obliquely striped. Other indications as in Figs. 1—2, 13. 90 ×.

an outward curve of the trabecular-basiotic laminar bar (Fig. 16). In this connection it can be noticed especially that the opthalmic artery (a.oph.) no longer branches off from the carotis interna but is now directly continuos with the efferent pseudobranchial artery (Fig. 21, e.ps.a.); this vessel is loosing the connection with the carotid.

In the chordal parts of the skull it is of interest to notice (1) that the notochord (Fig. 16, ch.) is not the dominating element any more, its tip is now situated behind the hypophysis and the polar cartilages, (2) that the anterior parachordals (pch.a.) are still blastemal, whereas the mesotic and the occipital ones (pch.m, pch.o.) have turned into cartilage, (3) that the lateral connection between the anterior and posterior otic cartilages (ca.a, ca.p.) has also chondrified and appreciably increased in width and (4) that the preoccipital (pr.o.) and occipital arches (p.o.) now take part in the covering of the caudomedial portion of the labyrinths.

The anterior end of the *notochord* has just begun to reduce (Fig. 17, ch.), a process that will proceed at the next stages. It leaves behind a canal, the posterior myodome (Fig. 34, p.my.), in which, as in *Amia* (Holmgren 1943; and others), only the external rectus muscles will insert (Fig. 35).

The chondrification of the mesotic and the occipital *parachordals*, which began at the foregoing stage, has continued. They are still possible to distinguish, however, as the chondrocytes are differently arranged in the two portions. Like the anterior parachordals the mesotic ones have now joined above the notochord.

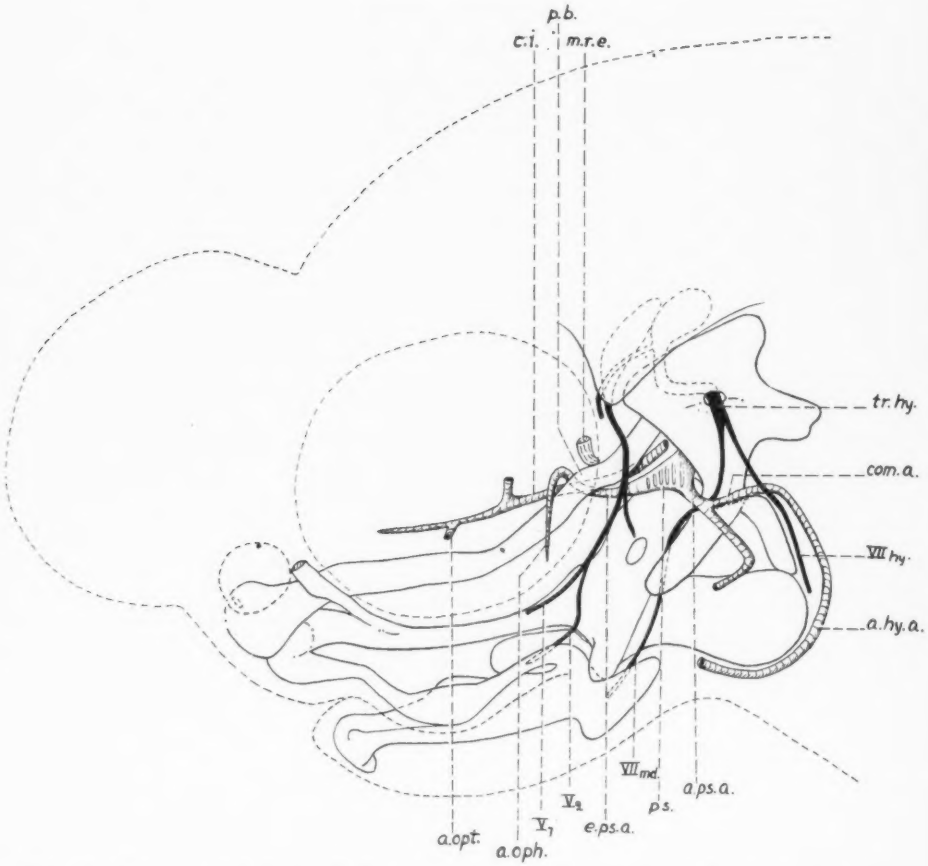

Fig. 18. *Hepsetus odoë*, 8 mm B. Reconstruction of anterior part of chondrocranium to show the position and course of arteries (striped) and visceral nerves (black). Lateral view. 69 ×.

The anterior basicapsular and the basivestibular commissures (a.bc.c., bv.s.) have increased reducing the basicranial foraminae (fe.b.$_{1,2}$) to a fraction of their earlier extention. An almost continuous basal plate has then been formed under the mid and hind brain. Because of different arrangement of their chondrocytes the different components of the plate (pch.a., pch.m., pch.o.; l.bas.a., l.bas.m., l.bas.o.) are, however, surprisingly well detachable from each other (cf. Fig. 19).

Of the *otic cartilages* especially the anterior ones have enlarged. They have continued enveloping the anterolateral part of the labyrinths, whereas the posterior ones are rather inactive and caudally only reach the crus commune. The covering of the posterior part of the labyrinths is instead achieved by the *preoccipital* and especially by the *occipital arches* (Figs. 15, 16, pr.o., p.o.). These have

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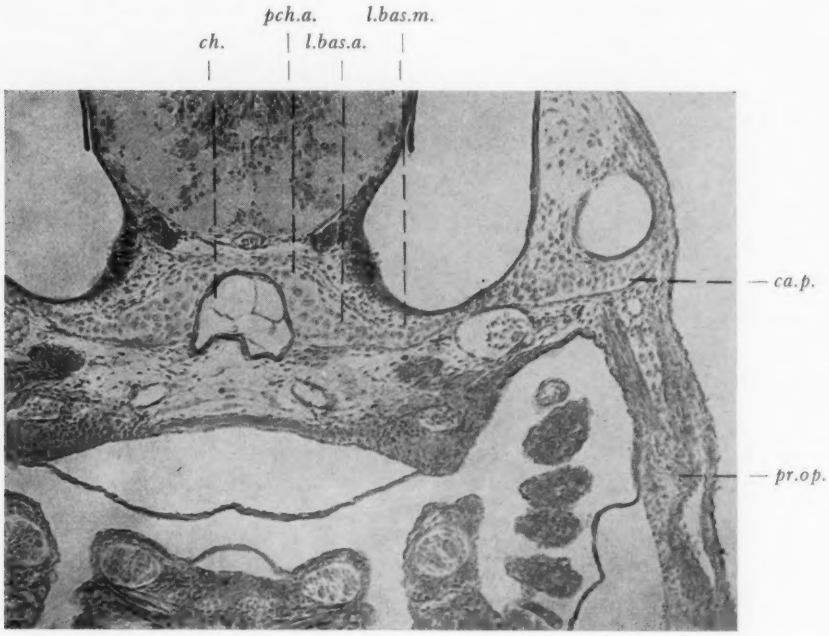

Fig. 19. *Hepsetus odoë*, 8 mm B. Transverse section through right part of skull to show the position of the anterior part of parachordals and basiotic laminas. 150 ×.

grown vigorously, extending forwards between the hindbrain (medially) and the posterior semicircular canals. Rostrodorsally they have even almost reached contact with the anterior otic cartilages (Fig. 15). Ventrally they are chondrifying. The preoccipital arches are also turning into cartilage. Dorsally they have fused with the occipital arches. The limit between them is, however, still discernible, partly morphologically as the n.vagus (X) issues between them, and partly histologically according to the different arrangement of their chondrocytes. The preoccipital arches constitute the ventromedial wall of the posterior semicircular canals (c.f. sharks, HOLMGREN 1940) as well as the mediodorsal wall of the prospective lagena (Fig. 28).

The strong differentiation of the covering of the labyrinths is preceded and accompanied by the differentiation of the otic vesicles. Already at the foregoing stage the semicircular canals were forming, and at this and the next stages also the sacculi and the lagenas are developing. Accordingly, it seems acceptable that the differentiation of the otic vesicles also in *Hepsetus* is the intrinsic factor for the vigorous development of the cartilage covering of the labyrinths that takes place at this and the next stages. This has been convincingly shown by experiments in *Ambystoma* (VAN DYKE & DETWILER 1958).

Finally, there is another element in the skull floor to be taken into account,

viz. the *lateral commissures* (Fig. 16, l.c.). They have grown in breadth and enclosed the r.palatinus facialis (VII). Further is to note that the lateral commissures are chondrifying in both ends. Yet, at this as well as at next stages they are histologically and morphologically discernible from the anterior basiotic laminas. The new connection with the anterior basicapsular commissure is on the contrary very intimate.

So far the differentiation of the neural cranium has been limited principally to the otic and occipital regions as well as to the basal parts of the orbitotemporal and the ethmoidal regions. However, at this stage, the dorsal parts of the latter regions have also begun to differentiate. Above the eyes and on each side of the diencephalon a prochondral cartilago supraorbitalis anterior (VEIT 1902) (Figs. 15, 16, c.so.a.) is formed. This element is sending out blastemal processes in three directions. Posteriorly a process forms, here called the *taenia marginalis postorbitalis* (t.m.po.), as it caudally meets the postorbital process (pr.po.) known from earlier stages. These two processes will later fuse and chondrify as the taenia marginalis posterior (SWINNERTON 1902, UHLMANN 1921, and others) (Fig. 26, t.m.p.). Medially is a process forming with its mate from the other side the epiphysial bar (Fig. 15, ep.b.); this is situated above the brain immediately posterior to the epiphysis. Rostrally there is the taenia marginalis anterior (t.m.a.) (SWINNERTON 1902). Anteriorly to the eye it is connected with a thicker blastema, the cartilago supraentethmoidalis (HAMMARBERG 1937) (ca.se.).

Each cartilago supraentethmoidalis has dorsally a short process, which, according to its position, seems to represent a reduced paraphysial bar (HAMMARBERG 1937) (pa.b.). However, as a taenia medialis is absent, it will never form a real bar but is later incorporated in the cartilago supraentethmoidalis. Further, this cartilage is medially connected with (1) the taenia marginalis ethmoidalis (t.m.e.) (the sphenoseptal commissure, DE BEER 1937), a membranous blastema situated dorsally to the nasal sac (Fig. 15, na.s.), (2) the processus entethmoidalis (pr.en.), a narrow blastemal process located medially to the nasal sac, and (3) via the sparsely nuclified blastema of the sphenethmoid commissure (DE BEER 1937) (s.c.) with the more differentiated blastema of the lamina orbitonasalis (DE BEER 1937; processus ectethmoideus; processus preorbitalis ALLIS 1897) (l.o.n.) posteriorly to the nasal sac. The processus entethmoidalis does not reach the trabecle as in *Holostei* (HAMMARBERG 1937), *Corydoras* (HAMMARBERG 1937), *Amiurus* (KINDRED 1919) and embryos of *Clupea* (WELLS 1922). However, according to HAMMARBERG (1937) it is missing altogether in other teleosts. Ventrally the processus entethmoidalis is connected with the m.obliquus superior (m.o.s.). Together with the m.obliquus inferior (m.o.i.) this muscle inserts on the trabecle. This place of insertion lies in the posterior part of the cavum orbitonasale (ALLIS 1897) (Fig. 15, c.on.), the short canal between the trabecle, the processus entethmoidalis and the lamina orbitonasalis. The posterior part of the cavum orbitonasale serves accordingly as an anterior eye muscle canal or ante-

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rior myodome (Fig. 16, a.my.). A well formed anterior myodome is present in most teleosts (*Clupea, Salmo* etc.; cf. HAMMARBERG 1937) but in *Amia* it is short (PEHRSON 1922) and in the silurids (POLLARD 1895, KINDRED 1919) and *Lepisosteus* (HAMMARBERG 1937) it is only diminutive.

Laminae orbitonasalia do not seem to develop from any separate blastema as in selachians (HOLMGREN 1940) and in certain teleosts, e.g. *Syngnathus* (KINDRED 1921, NORMAN 1925) and *Cyclopterus* (UHLMANN 1921). However, their ontogeny in Hepsetus does not either support the theory of DE BEER (1927), HAMMARBERG (1937) and others, that they arise from the trabecles. Instead their fusion with the trabecula communis is very thin (about 10—15 micrones) (Fig. 16), whereas they are broadly and intimately fused with the processus pterygoidei (Fig. 15, pr.pt.), indicating a primarily visceral origin. Furthermore, in two slightly younger specimens the orbitonasal laminas have no dorsal connection with the sphenethmoid commissure or the cartilago supraentethmoidalis. In these specimens as well as at the 8 mm stage the part of them that is best differentiated is situated caudally to the nasal sacs and broadly connected with the pterygoid processes.

Visceral cranium

All the visceral skeletal arches have now met ventrally and have become strongly differentiated (Figs. 20, 21). The deepening of the head fold and the upward turn of the neural cranium anterior to the polar cartilages (cf. p. 229) has made it possible for the ventral parts of the arches to grow forward and for the palatoquadrates to withdraw from the trabecles. The mandibles have almost reached the snout.

In the *mandibular arch* the pars quadrata is still protochondral but has changed morphologically. The processus basalis (Fig. 15, pr.bas.) has lost its dorsal blastemal connection with the polar cartilage simultaneously with the straightening of the neural cranium (cf. p. 229); it is, however, still facing the direction of the polar cartilage region. It has turned protochondral like the rest of the pars quadrata. Of the processus oticus externus (pr.ot.e.) only the ventral part is left. This has chondrified and according to the active growth of the caudomedial part of the pars quadrata it has attained a more posteroventral position. The foramen palatoquadratum (fo.pq.) is enlarged and situated immediately dorsally to the rest of the processus oticus externus. Dorsally to the foramen another process is appearing (pr.ot.i.). It is primarily situated inside the m.constrictor mandibularis dorsalis (m.levator arcus palatinus) and should be homologous with the *processus oticus internus* in selachians (HOLMGREN 1943). This process according to HOLMGREN corresponds to that one, which in fishes usually is called processus oticus.

In the mandible the processus coronoideus (pr.co.) has separated from the pars quadrata but is rostrally still connected with the maxillary and dentary blastemas, in the centre of which osteocytes are forming (mx.,de.). The dentary—which is

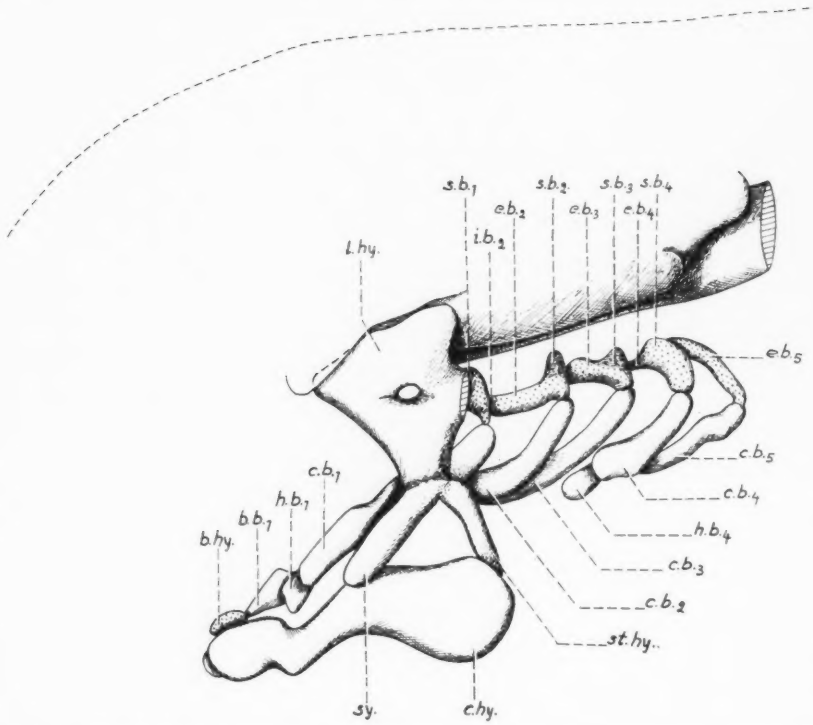

Fig. 20. *Hepsetus odoë*, 8 mm B. Reconstruction of visceral chondrocranium, lateral view. Mandibular arch omitted. Indications as in Figs. 1—2, 13. 69 ×.

not yet in contact with any sensory organ—is caudally covered by the angular blastema (ang.bl.). The posterior part of the meckelian cartilage (M.c.), the processus retroarticularis (pr.ra.), has chondrified and the former blastemal connection with the ceratohyal (cf. p. 227) is now ligamentous.

Anteroventrally the pars quadrata has, without any demarcation, fused with the processus pterygoideus (pr.pt.). However, a furrow in the cartilage marks the former limit against the basal process (Fig. 15). The pterygoid process is chondrifying from behind. Rostrally the former single connection with the trabecle is divided in two: one ethmopalatine connection with the lamina orbitonasalis and one rostropalatine with the ethmoidal plate. No articulations are, however, yet formed. The anterior end of the pterygoid process, the intermediating body (int. b.), is at this stage not only thicker than the rest of the process but also demarcated from it by means of a more sparsely nuclified blastema (Fig. 17). It is further discernible from the trabecula communis both histologically and morphologically.

The *hyal arch* is now chondrified except for some peripheral blastemal growing

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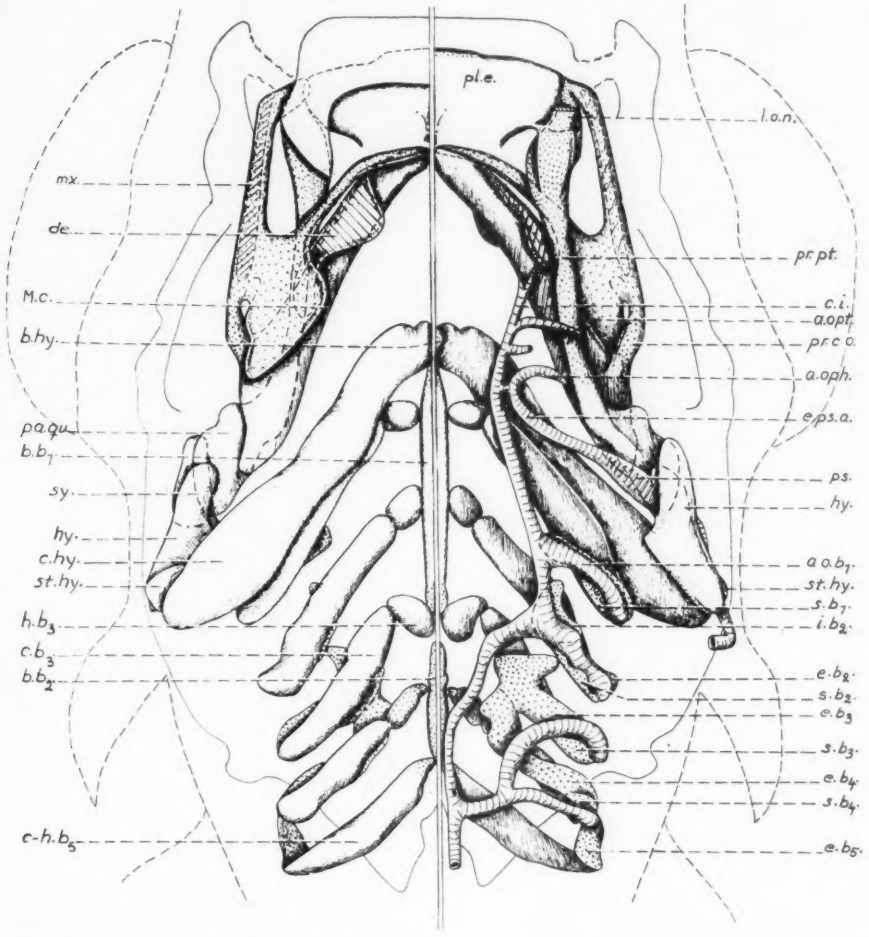

Fig. 21. *Hepsetus odoë,* 8 mm B. Reconstruction of visceral chondrocranium. Left, ventral view; right, dorsal view. The position and course of principal arteries shown on the right. Indications as in Figs. 1—2, 18. 69 ×.

zones (Fig. 15). The hyomandibula (hy) is almost unaltered. A morphological limit between its dorsomedial and dorsolateral (the laterohyal) portions is still seen on both sides of the foramen hyomandibularis (fo.hy.). The m.adductor hyomandibularis (differentiated from the m.constrictor hyalis dorsalis) inserts on the medial part of the hyomandibula just below the foramen. The opercular process (pr.op.) is large and rounded. In its lateral part the opercular bone (op.) is differentiating. Finally, it may be noted that the posterior articular head (p.ah.) has separated from the opercular process.

The sympletic (sy) is still histologically and morphologically discernible from

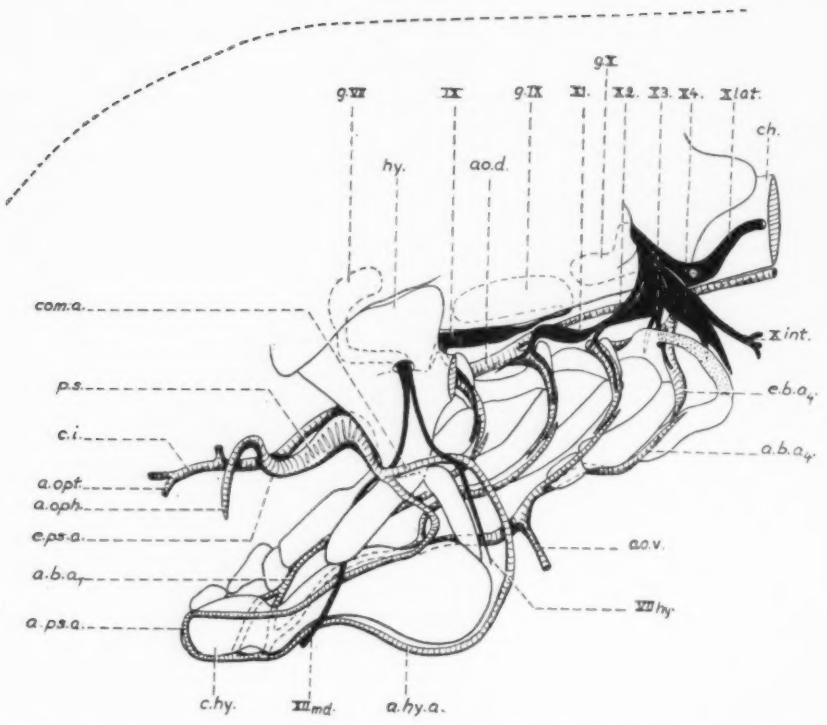

Fig. 22. *Hepsetus odoë*, 8 mm B. Reconstruction of visceral cranium except mandibular arch to show the position and course of visceral arteries (striped) and nerves (black). Lateral view. 69 ×.

the hyomandibula and the stylohyal (st.hy.). The latter has grown dorsoventrad; the middle of it is protochondral but still blastemally connected with the symplectic and the ceratohyal (c. hy.).

The ventral part of the hyal arch has grown markedly in length (Figs. 20, 21). Rostrally the two ceratohyals are connected by a blastemal basihyal (b.hy.). This is also the connecting link with the first branchial arch, as it posteriorly is connected with the first basibranchial (b.b.$_1$). This is chondrifying and extends back to the third branchial arch. Behind this the blastemal second basibranchial (Fig. 21, b.b.$_2$) connects with the remaining two arches.

The *branchial arches* have started to segment. In all arches except the fifth the anteroventral part of the ceratobranchials (Figs. 20, 21, c.b.) are chondrifying as hypobranchial elements (h.b.). Dorsally the prochondral epibranchials (e.b.) have grown still more antero-mediad. In the anterior part of the second epibranchial an infrapharyngeal element (i.b.$_2$) is chondrifying separately. In the others —all except for the fifth—similar independent chondrifications appear later. The

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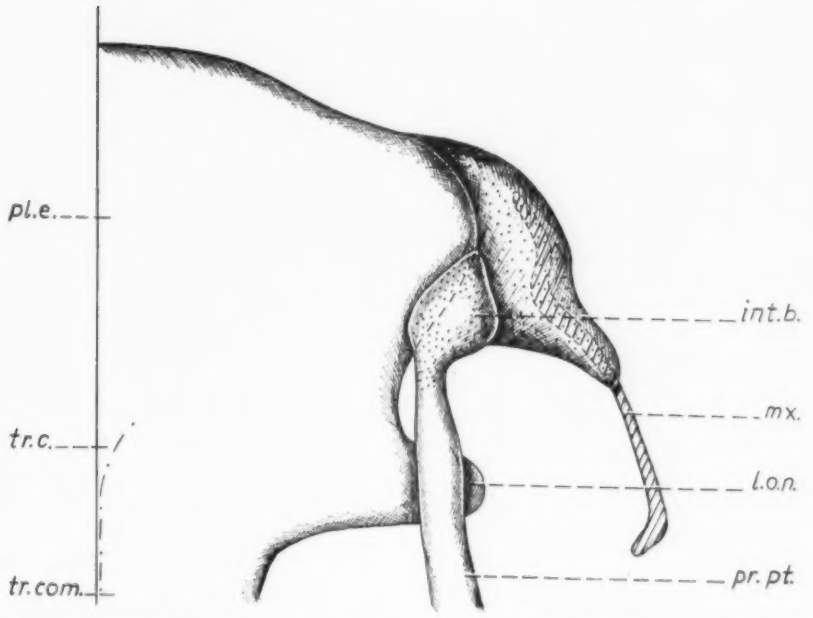

Fig. 23. *Hepsetus odoë*, 9 mm. Reconstruction of ethmoidal region to show the position of the intermediating body, ventral view. Indications as in Figs. 1—2, 18. 220 ×.

suprapharyngobranchials are still blastemal (s.b.). Their relations to the branchial nerves and vessels can be seen from Figs. 21 and 22.

Stage V, 9 mm (Figs. 23—24).

This stage is in its essential respects very much like the former. However, an important morphological and histological differentiation of the ethmoidal and orbitotemporal regions has made it necessary to describe it as a special stage.

Neural cranium

The ethmoidal region is characterized by a vigorous rostral growth especially of the ethmoidal plate (Fig. 23, pl.e.), whereas the trabecula communis (tr.com.) and the trabecular commissure (tr.c.) are rather unchanged. The lateral cornua of the ethmoidal plate are connected with the big intermediating bodies (int.b.), whereas the trabecula communis is now laterally broadly fused with the laminae orbitonasales (l.o.n). The nasal sacs are situated in front of these laminas and by a lengthened tractus olfactorius connected with the brain. The two nasal sacs are separated by a septum nasalis growing backwards from the taeniae marginales ethmoidales.

The splitting of the single connection between the palatoquadrates and the

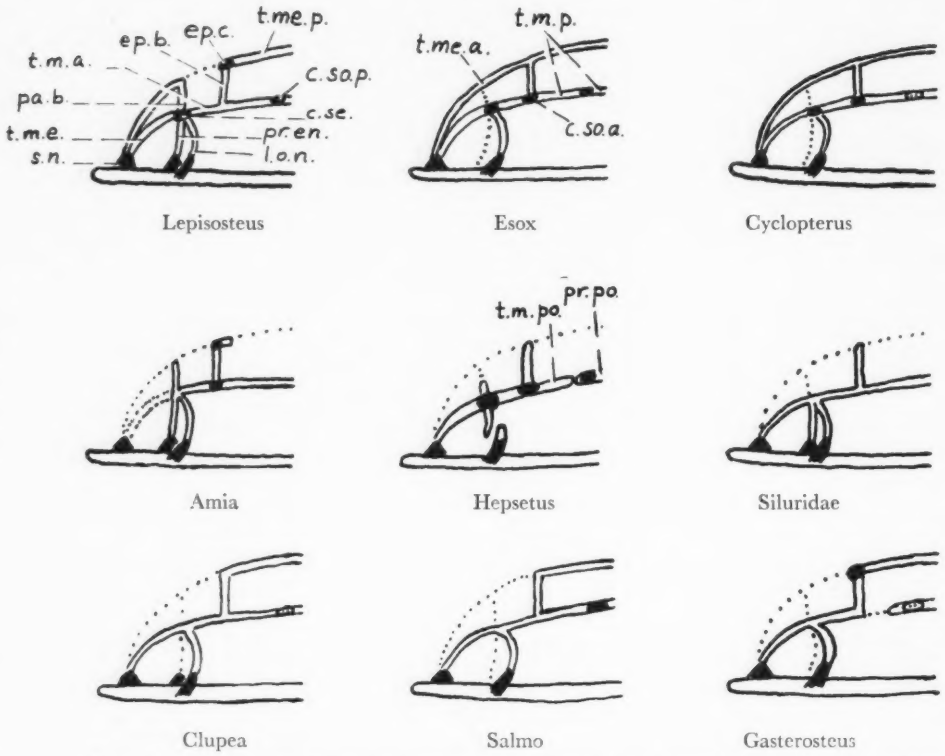

Fig. 24. Diagrams illustrating the forming of the neural chondrocranium in actinopterygians, lateral aspect. Chondrification centers black. Dotted areas and lines indicate reduced elements. All types except *Hepsetus* are from HAMMARBERG (1937, Fig. 38), partly modified.

ethmoidal region, which started in stage IV, has continued. No real articulations, however, have yet appeared. As to the intermediating body it is interesting to state its relative independence in relation to the rest of the pterygoid process (pr.pt.). It appears as a large knob in which separate chondrification has started.

Finally, there is to be mentioned that from this stage each cartilago supraentethmoidalis (cf. Fig. 24, c.se.) has chondrified and that the chondrification of the taenia marginalis ethmoidalis (t.m.e.), the sphenetmoid commissure (s.c.) and the adjacent part of the taenia marginalis anterior (t.m.a.) on each side emanates from this center. In the same way also each cartilago supraorbitalis anterior (c.so.a.) chondrifies and acts as a chondrification center for the adjacent parts of the taenia marginalis anterior, the taenia marginalis postorbitalis (t.m.po.) and the epiphysial bridge (ep.b.). There is, finally, a third chondrification center, a cartilago supraorbitalis posterior (c.so.p.), situated in the anterior part of the postorbital process just where it meets the taenia marginalis postorbitalis.

The organization of the dorsal part of the ethmoidal and the orbito-temporal

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regions in teleostomes has been studied particularly by HAMMARBERG (1937). To his schematic figure of the different types that of *Hepsetus* has been added in Fig. 24. It shows that in *Amia, Hepsetus* and the silurids the development of the ethmoidal region shows an intermediate type between the well developed type in *Lepisosteus, Esox* and *Cyclopterus* and the rudimentary type in *Clupea, Salmo* and *Gasterosteus.*

Stage VI, 12.6 mm (Figs. 25—32).

Looking at the outside of the head (Fig. 25) three facts can be noticed: the reduction of the adhesive organ, the development of teeth and, finally, the extension of the mandible beyond the snout. These external morphological signs indicate that the former larva now has developed into a complete free-living fish hunting for its food.

Internally the essential parts of the chondrocranium have formed and reached their largest relative size. Perichondral ossification has begun. However, some chondral growing zones are still left, indicating further extension particulary in length. Chondrification and ossification may accordingly occur simultaneously at this stage.

Neural cranium

This is characterized by the basal parts being straightened and by the vivid chondrification of the otic region. The straightening of the basal parts has ended at this stage; the trabecles (Fig. 25, tr.) are now situated on a level with the polar cartilages (p.b.) but have begun to separate from them. The labyrinths are completely differentiated and are covered laterally and dorsally by cartilage. Towards the brain, however, they are open, as is usually the case in teleosts.

The *trabecles* are anteriorly growing in thickness and subsequently the trabecula communis (Fig. 26, tr.com.) is increasing in length. The basicranial fenestra (f.b.) is then diminishing.

Rostrally the large ethmoidal plate (pl.e.) has grown still more rostrad and now extends far beyond the nasal sacs (Fig. 25). On the dorsal side it is, by means of the low nasal septum (s.n.), connected with the taeniae marginales ethmoidales (t.m.e.) and the entethmoidal processes. These have become incorporated into the nasal septum and are not recognized any more. Since the laminae orbitonasales (l.o.n.) have grown in thickness and become broadly chondrally fused to the trabecular commissure, the cavum orbitonasale (c.on.) has been strengthened on two sides, laterally and medially. The cavum is rostrally continued by the fossa nasalis (fo.n.), a rostrocaudal groove between the ethmoidal plate, the nasal septum and the taenia marginalis ethmoidalis. The tractus olfactorius runs through the cavum orbitonasale and then through the fossa nasalis before it reaches the nasal sac. The m. obliquus superior and m. obliquus inferior

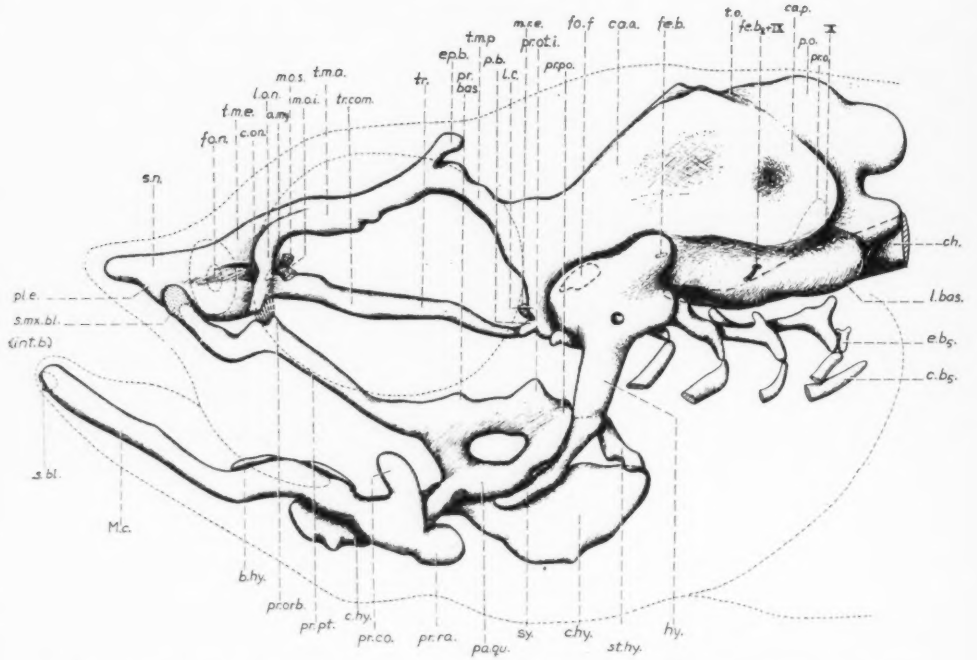

Fig. 25. *Hepsetus odoë*, 12.6 mm. Reconstruction of chondrocranium, lateral view. The cranium is chondrified except for the intermediating body. Indications as in Fig. 13. 41 ×.

(m.o.s., m.o.i.) insert as before in the posterior part of the cavum (cf. p. 234); this cavity is the anterior eye muscle canal or anterior myodome (a.my.).

The dorsal frame of the eyes is completely chondrified and no chondrification centers can be seen any more.

The *polar cartilages* (pb.) are relatively small and partly changed. Rostrally their chondrocytes are breaking down dorsally to the efferent pseudobranchial artery (Fig. 27, e.ps.a.); a limit against the trabecles in thus re-established. Caudally the polar cartilages have grown in width and are more broadly connected with the anterior basiotic laminas (l.bas.a.). The polar cartilages at this stage are hence incorporating into the otic region.

The basal plate is almost completely chondrified exept for the small fenestrae (Fig. 26, fo.f., fe.b.$_{1,2}$). The notochord (ch.) is still more reduced, the tip now only reaching the level of the anterior basal fenestrae. The rostral part of the fused anterior *parachordals* (pch.a.) is the only part of the cranial base that is still blastemal. Posteriorly, however, they have chondrified and form a narrow strip of cartilage between the notochord and the anterior basiotic laminas (l.bas. a.). The mesotic parachordals (pch.m.) are resorbed rostrally giving room to the ductus communicans transversus of the sacculi; posteriorly the mesotic para-

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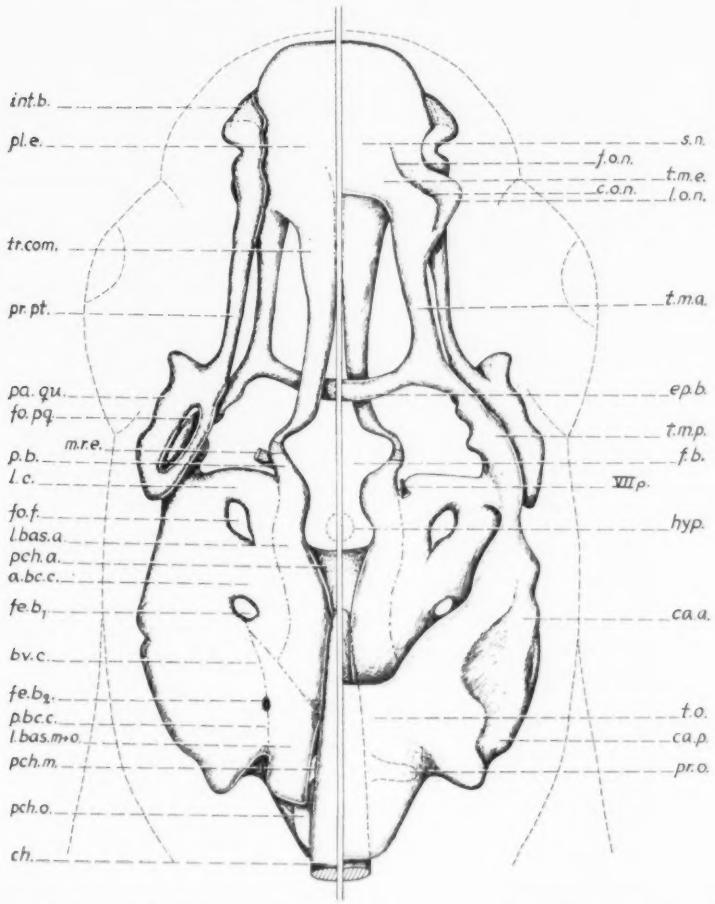

Fig. 26. *Hepsetus odoë*, 12.6 mm. Reconstruction of neural chondrocranium and palatoquadrates. Left, ventral view; right, dorsal view. Indications as in Fig. 13. 41 ×.

chordals extend on the medial side of the lagenas. A weak chondrification zone separates the mesotic and occipital parachordals (pch.o.). The ventral and lateral sides of the lagenas are covered by the fused mesotic and occipital *basiotic laminas* (Fig. 28 l.bas.m.+o.).

Dorsally the preoccipital processes (Figs. 25, 26, pr.o.) retain the same position but are completely chondrified. The posterior *otic cartilages* (ca.p.) have begun to grow laterodorsad outside the posterior part of the labyrinth, dorsally fusing with the occipital arches (p.o.). These have fused medially and connected with the anterior otic cartilages (ca.a.). In that way a roof has been formed above the hind brain, the tectum synoticum (t.o.) and continuous cartilaginous otic capsules envelop the labyrinths from the lateral side.

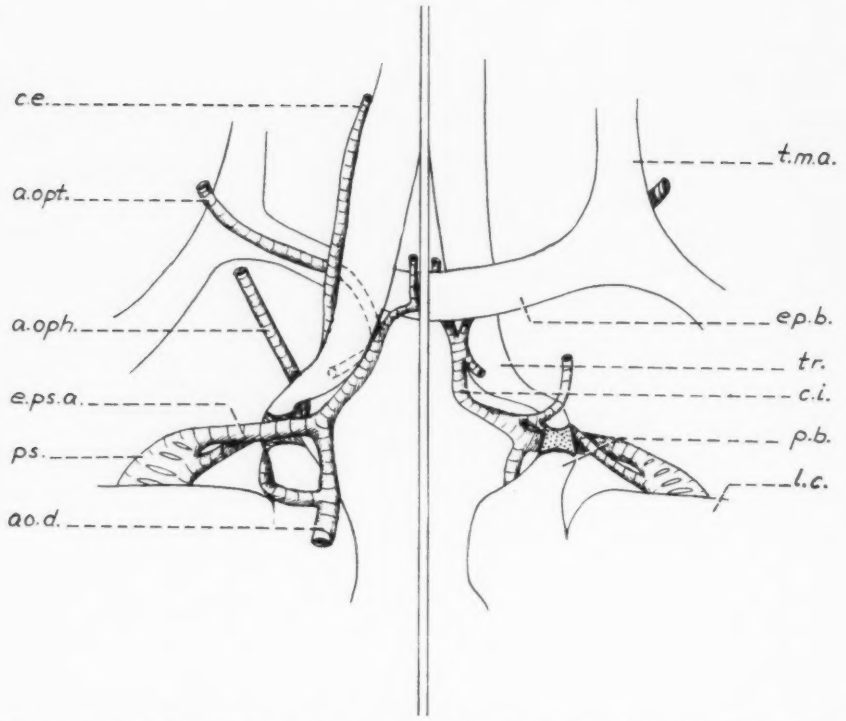

Fig. 27. *Hepsetus odoë*, 12.6 mm. Reconstruction of part of neural chondrocranium showing the position and course of principal arteries. Lateral aspect. 96 ×.

The lateral commissures (l.c.), finally, have chondrified except for the part anterior to the rr.palatini facialis (Fig. 26, VIIp) that instead seems to have become dissolved. From their original position caudally to the lateral commissures the rr.palatini hence emanate anterior to the commissures at this stage.

According to DE BEER (1937) the lateral commissures are formed from three different neurocranial processes: the prootic process from the otic cartilages, the postpalatine process from the parachordals, and the basitrabecular process from the polar cartilages. As a basis for this opinion he refers to investigations of *Amia* (PEHRSON 1922), *Lepisosteus* (HAMMARBERG 1937) and *Salmo fario* 14 mm and *S. salar* 25 mm and 35 mm (DE BEER 1927). Obviously, however, the "processes" described by PEHRSON and HAMMARBERG referred to the *chondrification* of the lateral commissures* (cf. *Hepsetus* stage IV.). Also in *Salmo* the "processes" seem

* In *Amia* the uniform lateral commissures later secondarily envelop the rr.palatini (cf. *Hepsetus* stage IV), whereas in *Lepisosteus* the later ontogeny is confused. HAMMARBERG'S description of stage II (11 mm) does not tally with his fig. 4 of the stage. The first (chondral) rudiment of the lateral commissure is said to be the "Commissura postpalatina", but his fig. shows that this commissure also joins the polar cartilages. The rr.palatini hence pierce the "Commissura postpalatinas" instead of running anteriorly to them as the name implies.

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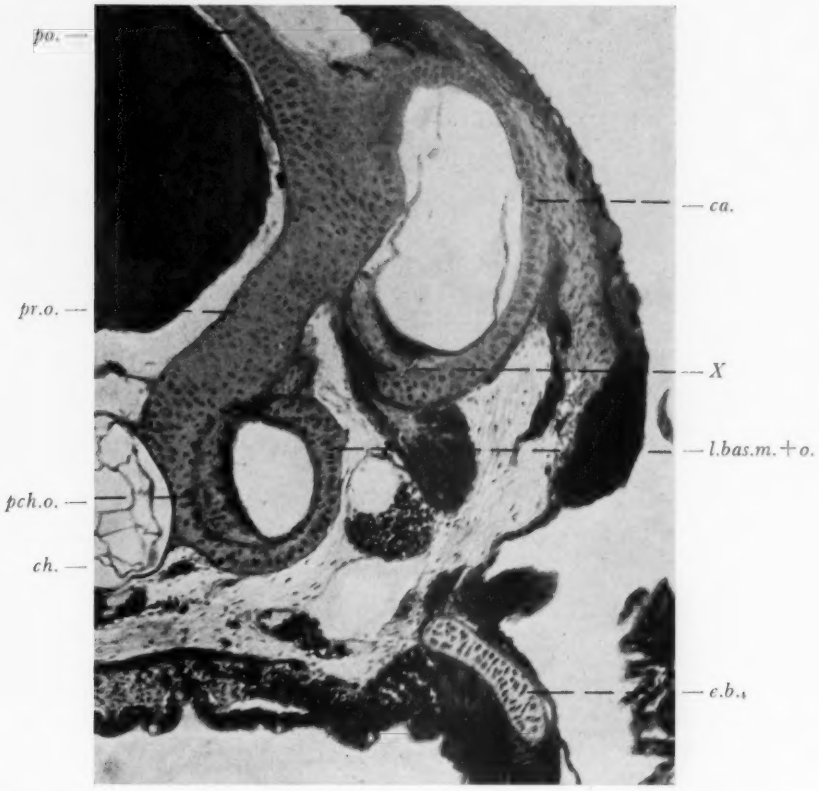

Fig. 28. *Hepsetus odoë*, 12.6 mm. Transverse section through right part of the occipital region of skull. The section shows the position of the occipital and preoccipital arches in relation to otic cartilage, parachordal and basiotic lamina. 160 ×.

to apply only to the extension of the chondral tissue, whereas DE BEER has mentioned nothing of the lateral commissures in the blastemal stage. Accordingly it is probable that the lateral commissures in *Salmo,* as in *Hepsetus, Leuciscus* (HUBENDICK 1943), *Gasterosteus* (SWINNERTON 1901/02) and probably most of the other teleosts, might have *blastemally* extended essentially from the otic cartilages (as the prootic processes). An examination of the *Salmo* material available at this Institute (cf. p. 205—206) strengthens this supposition.

However, if compared with *Neoceratodus* (p. 313) it seems probable that the lateral commissures of fishes originally were not formed from the neural cranium but from the visceral cranium (the hyal arches). A visceral origin of the lateral commissures has also earlier been suggested by comparative anatomists such as ALLIS (1914) and HOLMGREN (1943) (cf. chapter V).

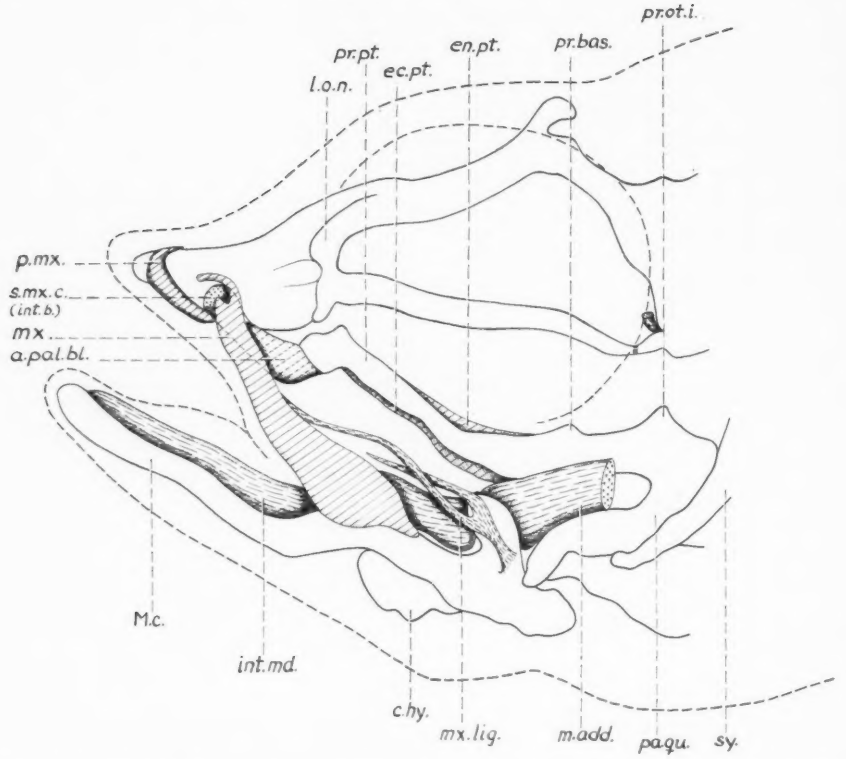

Fig. 29. *Hepsetus odoë*, 12.6 mm. Reconstruction of anterior part of skull to show the position of certain ligaments (wavy lined), muscles (sligthly waved) and bones (obliquely striped) in relation to the chondrocranium. Lateral aspect. 51 ×.

Visceral cranium

The turning upwards of the anterior parts of the neurocranium is also in this stage accompanied by a growth in length of the ventral parts of the visceral arches (Fig. 25). This process is especially pronounced in the *mandibular arch*, where the meckelian cartilage (M.c.) now extends beyond the tip of the snout. The simultaneous ventrad movement of the pars quadrata, which started at the former stage, is ceasing at this stage. The pars quadrata (pa.qu.) now runs horizontally. Processus basalis (pr.bas.) is unchanged in size but has got a more frontal position because of the growth rostrad of the arch. In the mandibula the big coronoid process (pr. co.) is now only connected with the maxillary. The connection has differentiated into ligament (Fig. 29, mx.lig.). The original blastemal connection with the dentary has dissolved proximally; the rest has become ligamentous and joined the maxillary ligament (Fig. 29).

The former blastemal connection between the lamina orbitonasalis and the

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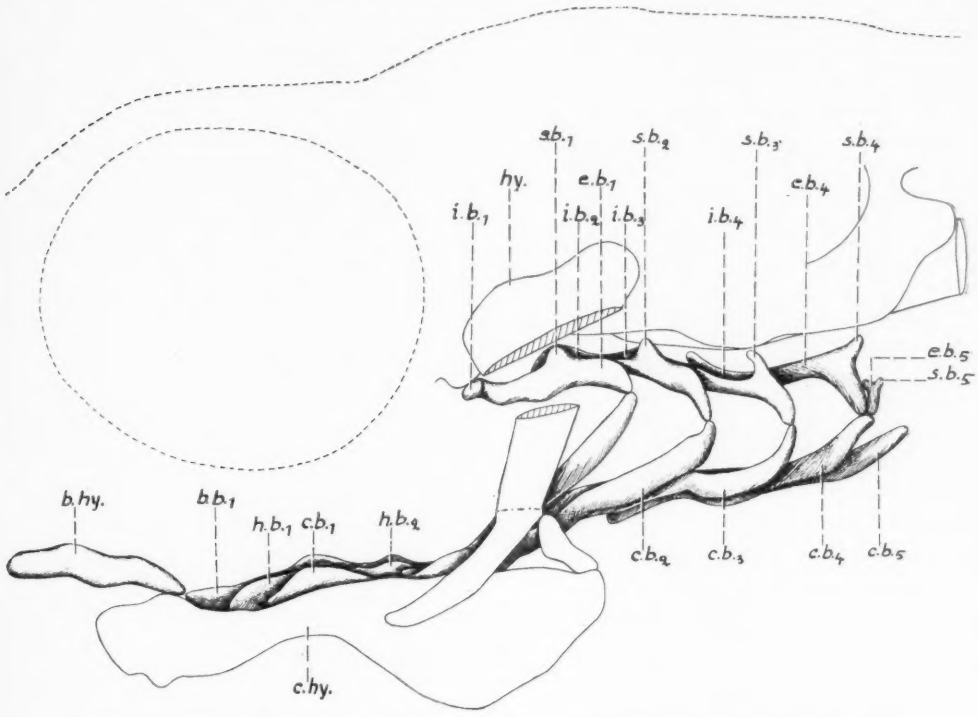

Fig. 30. *Hepsetus odoë,* 12.6 mm. Reconstruction of branchial arches, lateral view. 51 ×.

pterygoid process has differentiated into fibrocytes in its middle part and into chondrocytes in its basal part (Fig. 25). Hence a dorsally pointing chondral process (pr.orb.) is forming on the processus pterygoideus; this process later will articulate with the base of the lamina orbitonasalis in the ethmopalatine articulation. Such a process is formed also in other teleosts, e.g. *Esox* (WALTHER 1882), but it has hitherto not got any special name. In his thorough study of the embryogenesis of selachians, HOLMGREN (1940, 1943) has shown that the orbital process in these fishes is primarily formed posteroventrally to the lamina orbitonasalis by the breaking up of the ethmoidal connection between the trabecle, the lamina and the palatoquadrate. In his paper 1943 he points to this fact, and, discussing the homology of the ethmopalatine articulation in teleosts, he considers this articulation to be corresponding to "the orbital articulation in sharks". Of topographical reasons this seems very reasonable, and the homologization should hence be extended also to the processes. The process in question in *Hepsetus* and other teleosts should accordingly be called *processus orbitalis.*

The intermediating body in this specimen is still blastemal (int.b.). It can be noted in this respect that although the maxillary (Fig. 29, mx.) is blastemally

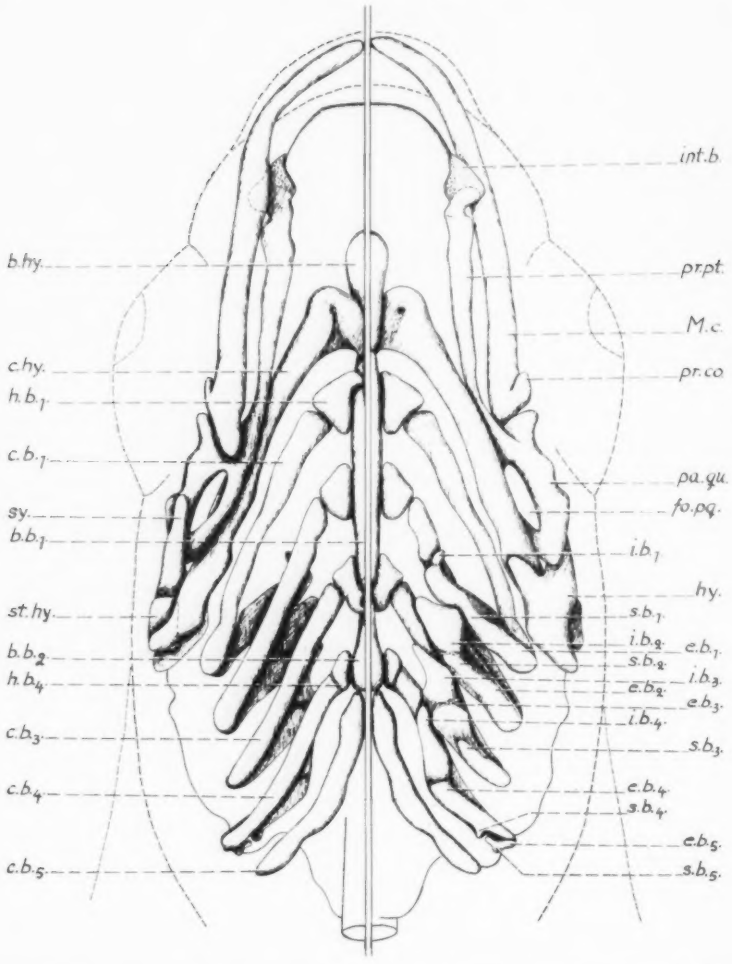

Fig. 31. *Hepsetus odoë*, 12.6 mm. Reconstruction of visceral chondrocranium. Left, ventral view; right, dorsal view. Neural chondrocranium is indicated by continuous lines without any shading. Indications as in Fig. 13. 39 ×.

connected with the lateral side of the intermediating body it has also a dorsal process connected with the lateral edge of the ethmoidal plate. Judging from the conditions in other characids this double attachment of the maxillary is of decisive importance for the question if the intermediating body differentiates into separate submaxillary cartilages (SAGEMEHL 1883) (cf. p. 342).

In the *hyal arch* the sympletic (Fig. 25, sy.) has extended rostroventrad simultaneously with the twisting of the mandibular arch. It is at all times free from the pars quadrata and connected with—though still histologically discernible

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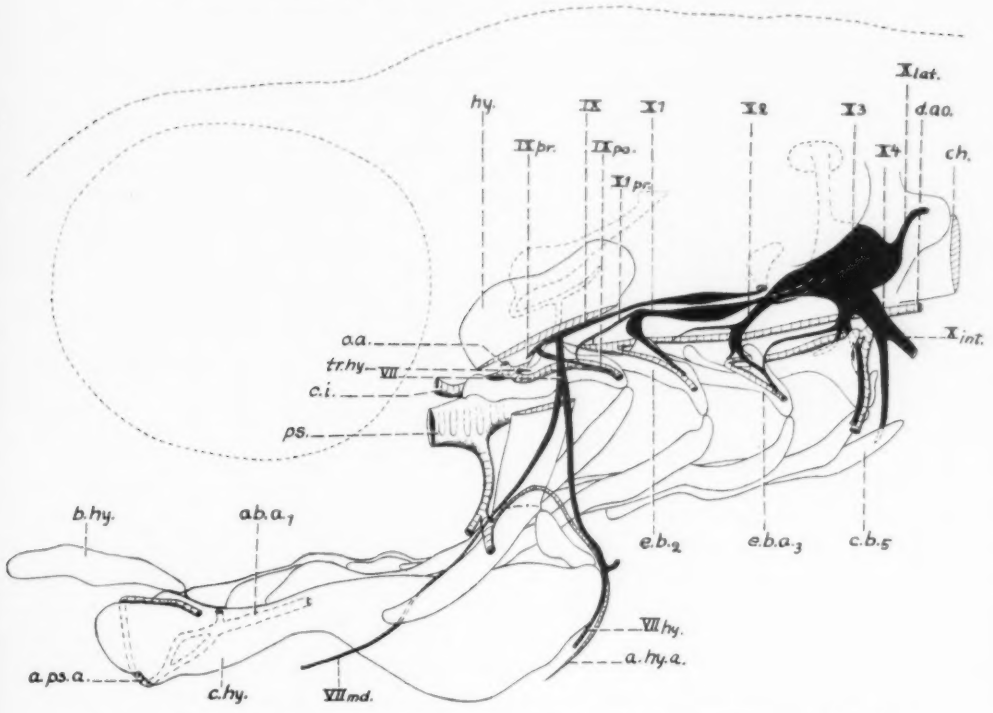

Fig. 32. *Hepsetus odoë*, 12.6 mm. Reconstruction of visceral chondrocranium (mandibular arch omitted) to show position and course of visceral arteries (striped) and nerves (black). 51 ×.

from--the hyomandibula (hy.). The stylohyal (st.hy.) has chondrified altogether and separated from the symplectic and the ceratohyal (c.hy.). The latter has grown forwards and now reaches almost the middle of the mandibula. Rostrally it has grown around the afferent pseudobranchial artery (Fig. 32), which hence pierces the ceratohyal now. The basihyal (Fig. 31, b.hy.) has chondrified. In the floor of the pharynx it caudally meets the large chondral basibranchial$_1$ (b.b.$_1$). This cartilage, in its turn, is caudally bordered by the chondrified, short basibranchial$_2$ (b.b.$_2$).

As to the rest of the *branchial arches* it should be mentioned that apart from the strong growth rostrad of the ceratobranchials (Figs. 30, 31, c.b.), the fifth epibranchial (e.b.$_5$) has chondrified and separated from its ceratal element (c.b.$_5$). Instead it has connected posteromedially with the fourth epibranchial (e.b.$_4$).

The branchial arches have now become definitely segmented. An infrapharyngobranchial (i.b.) has separately chondrified in each arch except the fifth. It is, however, still blastemally connected with the epibranchial. Laterally the second

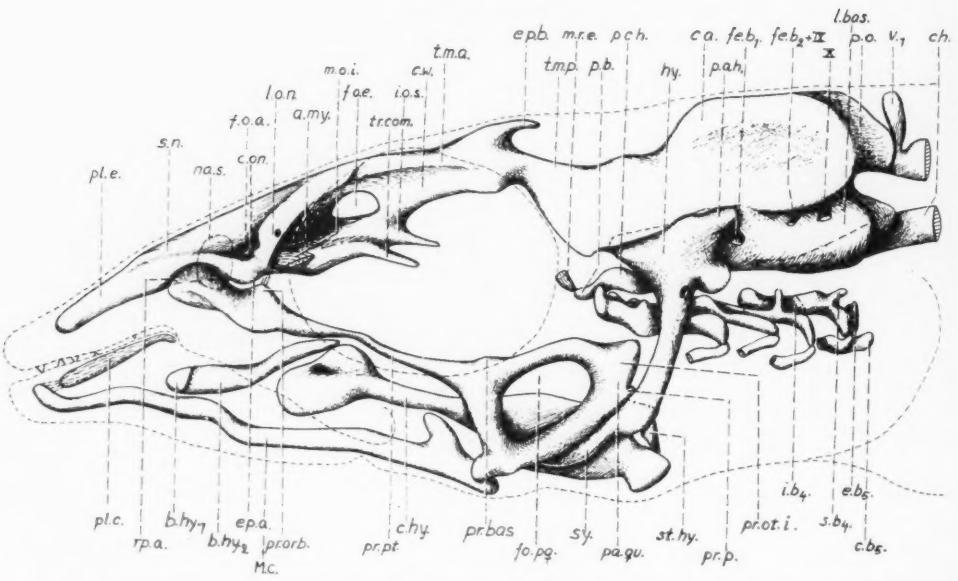

Fig. 33. *Hepsetus odoë*, 23 mm. Reconstruction of chondrocranium, lateral view. Dot-lining indicate histologically and morphologically different elements bound together by a common perichondral membrane. 25 ×.

and the third infrapharyngobranchials interarcually articulate with the base of the suprapharyngobranchial anterior to them (s.b.), whereas the fourth infrapharyngobranchial articulates with the epibranchial of the arch in front. The first infrapharyngobranchial is blastemally connected with the lateral margin of the lateral commissure.

The third and fourth suprapharyngobranchials have grown larger than the others. A rudimental fifth suprapharyngobranchial is present (s.b.$_5$). Such an element does not seem to be described in any other teleostome. It has probably remained in the characids to give support to the large posterior gills.

When considering vessels and nerves the fact can be mentioned that the afferent hyal artery—as is usually the case in teleost embryos (KRYZANOVSKY 1934; and others)—is vanishing (Fig. 32, a.hy.a.). The pretrematic rami of the branchial nerves stretch rostrad and join the post-trematic rami of the branchial nerves in front.

Stage VII, 23 mm (Figs. 33—37, 39—40).

The chondrocranium is now completely formed and the regression and the ossification are more accentuated. The head has attained adult appearance: an elongated skull (Fig. 33) with a strongly differentiated ethmoidal region and large jaws with many large canine-like teeth. The fish is now, in its essential respects, fit for a carnivorous life.

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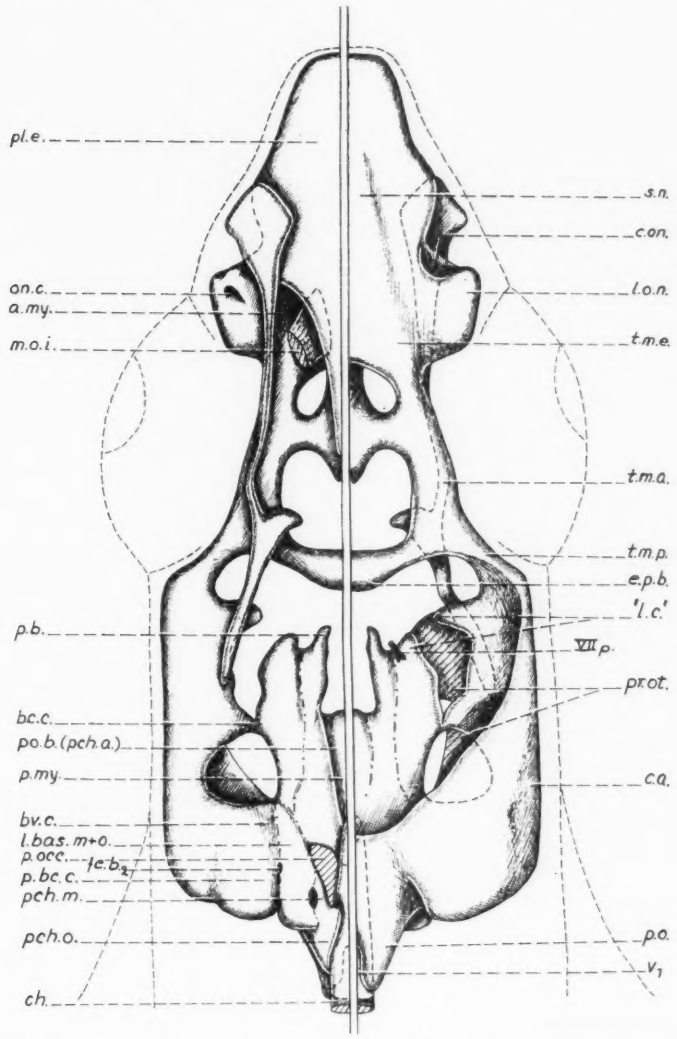

Fig. 34. *Hepsetus odoë*, 23 mm. Reconstruction of neural chondrocranium and palatoquadrates. Left, ventral view; right, dorsal view. Bones obliquely striped. Dot-lining as in Fig. 33. 25 ×.

Neural cranium

The neural cranium has grown in length still more (Figs. 33, 34). As at the two previous stages this applies mainly to the ethmoidal region. The growth concerns essentially the ethmoidal plate (pl.e.), which has rostrally formed a tip, extending far beyond the nasal sacs (na.s.). The laminae orbitonasales (l.o.n.) are situated between the nasal sacs and the eyes. They hence constitute the border

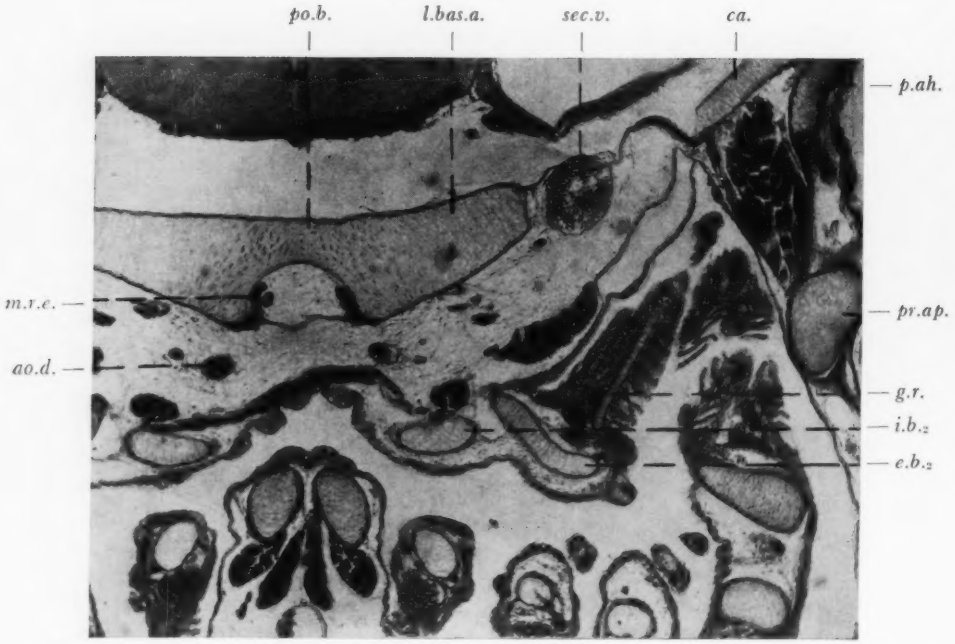

Fig. 35. *Hepsetus odoë*, 23 mm. Transverse section of skull to show basal plate, posterior myodome and dorsal gill rays. 125 ×.

between the ethmoidal and the orbito-temporal regions. The laminae have grown dorsolaterad and enclosed a branch of the orbitonasal artery in a short canal (on.c.).*

The connecting link between the two anterior regions is the nasal septum (s.n.). The brain has not taken part in the growth rostrad of the cranium, and the telencephalon now only reaches the middle of the orbit. The nasal septum has extended caudad and simultaneously a process has formed from each cartilago supraorbitalis anterior, the preoptic root (DE BEER) (pila prooptica). In the orbit an anterior medial wall is formed in that way (Fig. 33, c.w.). It is pierced by a foramen olfactorium evehens (f.o.e.) for the olfactory nerve. The nerve runs rostrad across the orbit, and accompanies the orbitonasal artery through the cavum orbitonasale (c.on.). It leaves through the olfactorium advehens (f.o.a.) immediately rostrally to the lamina orbitonasalis, and finally, as before, runs rostrad in the fossa nasalis (fo.n.) before joining the nasal sac.

The *trabecles* are resorbed caudally and only the trabecula communis (tr.com.) is left. The case is usually so in teleosts, e.g. *Clupea* (WELLS 1922), *Leuciscus*

* In *Leuciscus* (HUBENDICK 1943) and silurids (KINDRED 1919, SRINIVASACHAR 1957) the n.ophtalmicus profundus pierces the orbitonasal lamina in a canal of about the same position as in *Hepsetus*. This nerve, however, runs dorsally to the lamina in *Hepsetus* (Fig. 36).

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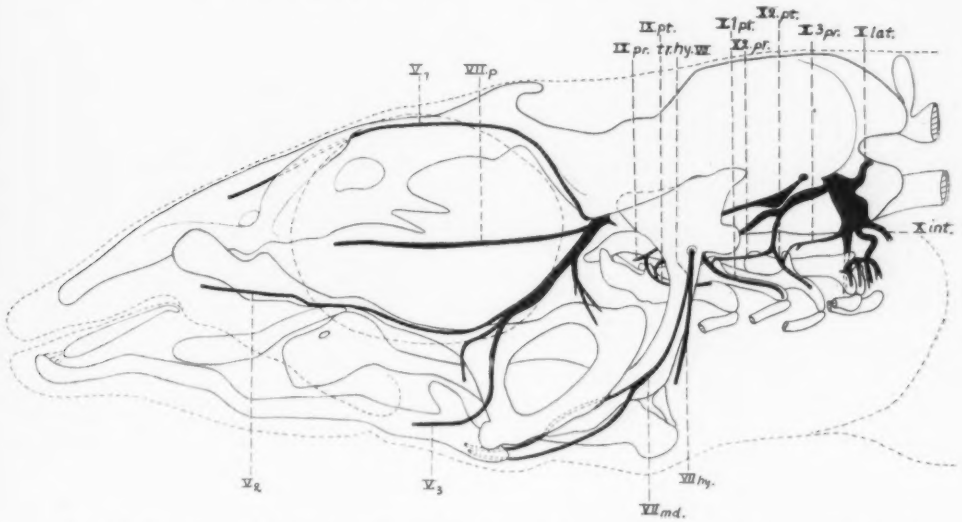

Fig. 36. *Hepsetus odoë*, 23 mm. Reconstruction of chondrocranium showing position and course of visceral nerves. Lateral view. 25 ×.

(Hubendick 1943), *Eleutheronema* (Marathe & Bal 1956), *Hippocampus* (Kadam 1958) and *Mastacembulus* (Bhargava 1958). The *polar cartilages* (p.b.) are then freed from the trabecles and have been incorporated into the otic region. The fissure between them and the lateral commissures (l.c.) is widened and lodges in addition to the r. palatinus facialis also the carotis interna.

The otic and occipital regions are characterized by an intense ossification process. A large part of the mesotic *parachordals* (pch.m.) has been resorbed and replaced by the basioccipital bone (b.occ.), whereas the middle of the lateral commissures and part of the anterior basicapsular commissures (a.bc.c.) have yielded place by the prootic bones (pr.ot.). The remaining part of the chondral neural skull is covered by a periosteal membrane. The former morphological demarcation lines between the chondral elements in the basal plate are not, however, hard to recognize, as the chondrocytes are usually arranged differently in the various elements and the intercellular substance in the demarcation zones is little developed (Fig. 35).

The tip of the notochord (Fig. 34, ch.) reaches the middle of the otic region. As the anterior parachordals have simultaneously chondrified completely, constituting the medial part of the prootic bridge (po.b.), there has hence been formed a triangular room. In this room the mm. recti externi are lodged. In this way *Hepsetus* has developed a posterior myodome (p. my.) of *Amia*-type (Holmgren 1943). Other characids also have the anterior rectus muscles lodged in it. As *Salmo* and most other teleosts investigated have a complete myodome containing

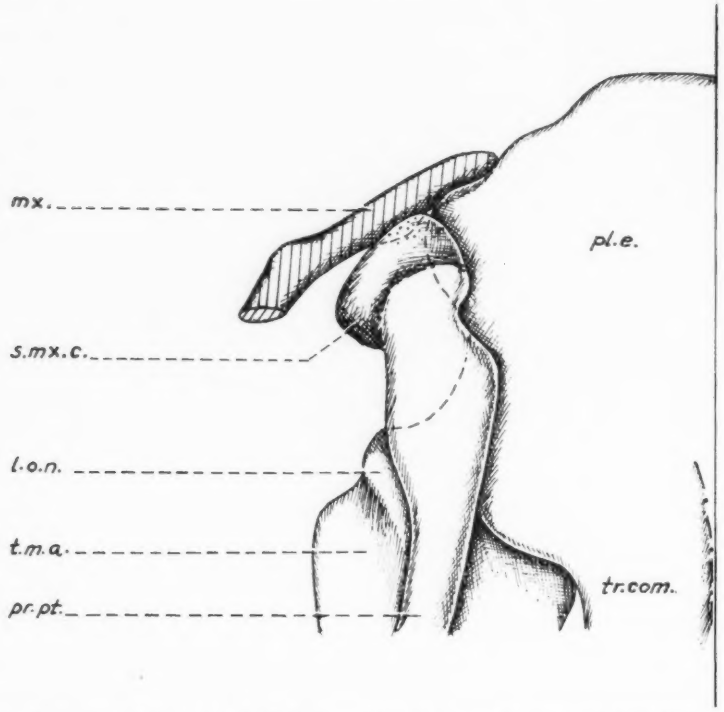

Fig. 37. *Hepsetus odoë*, 13 mm. Reconstruction of anterior part of chondrocranium to illustrate how the intermediating body (submaxillary cartilage) may show tendencies to separate from the tip of the pterygoid process (cf. Fig. 85 A, B). Ventral view. Indications as in Fig. 33. 62 ×.

all rectus muscle, the *Characidae* and especially *Hepsetus* are more like *Amia* in this connection.

Finally, there is to mention that the first vertebrae (v_1) are still chondral. They are connected with the portion of the otic cartilages (ca.) that is formed by the occipital arches (p.o.).

Visceral cranium

Also the visceral cranium is characterized by a certain reduction of cartilage, caused by an acceleration of the ossification process. The foramen palatoquadratum (Fig. 33, fo.pq.) for example, has been widened, which gives the ossifying pars quadrata (pa.qu.) the appearance that is so characteristic for the characidean *mandibular arch*. As to the pars quadrata, the internal otic process (pr.ot.i.) has grown caudad and now lies dorsally to the posterior process (pr.p.) and is only separated from this by the afferent pseudobranchial artery.

Each palatoquadrate has been strengthened rostrally since also the intermediat-

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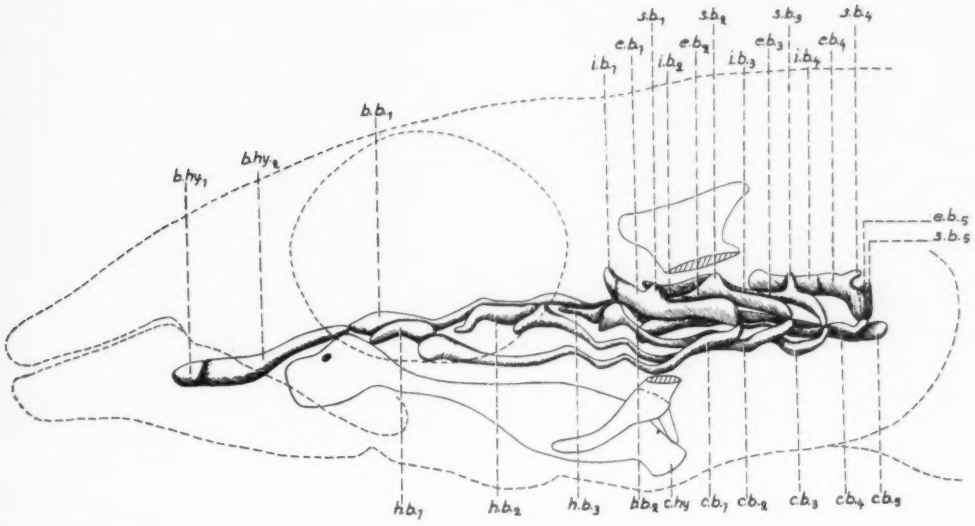

Fig. 38. *Hepsetus odoë*, 23 mm. Reconstruction of branchial arches, lateral view. 25 ×.

ing body (int.b.) has chondrified. This element sometimes shows tendencies to become separate (Fig. 37, s.mx.c.) and then to correspond to the submaxillary cartilage of other characids (p. 343).

Since the processus orbitalis of the palatoquadrate (Fig. 33, pr.orb.) now articulates with the neural cranium (the lamina orbitonasalis), *Hepsetus* has acquired two strong articulations anteriorly, an anterior rostro-palatine (rp.a.) and a posterior ethmo-palatine (orbital HOLMGREN 1943, antorbital SEWERTZOFF 1928) articulation (ep.a.).

In the lower jaw a long medial cord of fibrous cartilage, here called the *prelingual cord* (pl.c.), has formed. It is fibrously connected with the junction of the meckelian cartilages (M.c.) but is posteriorly free from the anterior basihyal cartilage (Fig. 33, $b.hy_1$). It does not ossify. The function seems to be to strengthen the mouth floor. No corresponding structure seems to be known in other recent fishes but the cord may perhaps correspond to the endoskeletal sublingual rod in *Eusthenopteron* (JARVIK 1954).

The *hyal arch* is ventrally connected with its mate by means of a long basihyal ($b.hy._2$). At the anterodorsal border of this, however, a flat *anterior basihyal* ($b.hy._1$) is located above the entoglossum. In a 13 mm specimen it consists of a blastemal pad at the tip of the chondral $basihyal_2$, and in a 43 mm specimen it has been chondrified separately anteriorly to the entoglossum. The element is also present in the other characids investigated but it seems not to be described in other teleosts. It never has any connection with muscles and it is anteriorly surrounded by elastic cartilage without any perichondral demarcation.

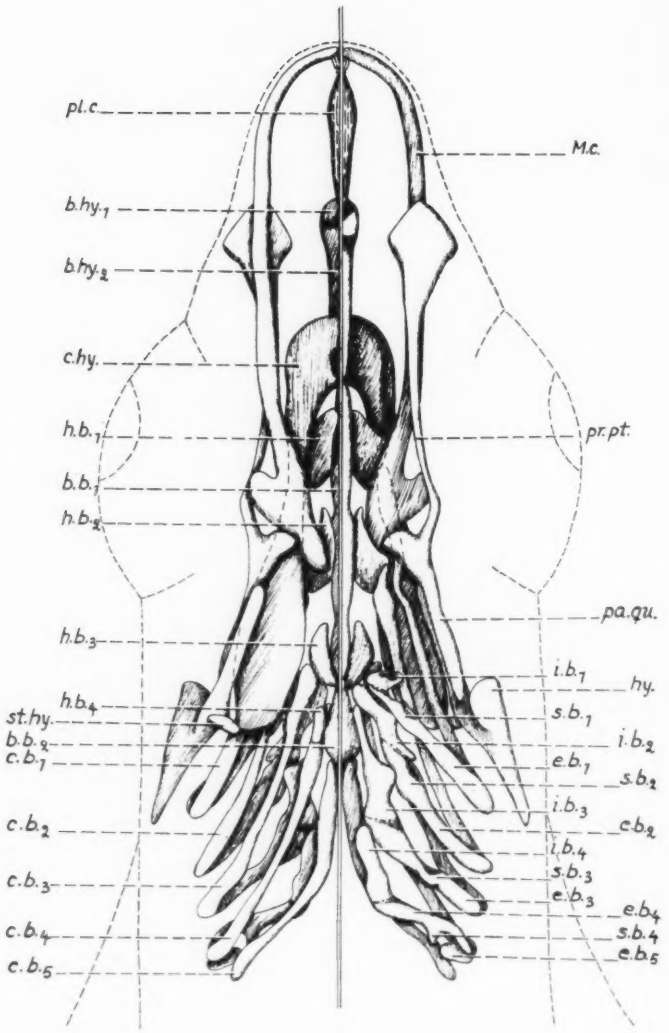

Fig. 39. *Hepsetus odoë*, 23 mm. Reconstruction of visceral chondrocranium. Left, ventral view; right, dorsal view. 25 ×.

In the hyomandibula the processus opercularis (Fig. 33, pr. op.) and the posterior articulating head (p.ah.) have been lengthened posteriorly and the distance between them has increased. Between them the subepidermal tissue is vascularized, forming an extrahyal venous plexus. According to SAGEMEHL (1884) certain characids have an opercular pseudobranch in their juvenile stages. In *Hepsetus*, however, no traces of such an element can be seen.

The basal parts of the *branchial arches* (Figs. 38, 39) have grown forward and

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are simultaneously ossifying. Otherwise they have not altered. Concerning the branchial nerves all pretrematic rami have now joined the posttrematic rami of the nerves in front of them (Fig. 36).

IV. On the chondrocranial base in fishes

In the neural cranium the development of the chondrocranial base (basis cranii) is of special interest seen from comparative anatomic as well as phyletic point of view, because the basis cranii is composed of elements that belong to the first differentiated skeletal parts of the head (cf. DEVILLERS 1958; and others): parachordals, basiotic laminas and notochord in the occipital and otic regions, polar cartilages and trabecles in the orbito-temporal region (the trabecles later extend up to the ethmoidal region). Problems of interest are for example, if there are traces of an original segmentation also of the teleostomian head, and if it is possible to discern visceral components in the neural cranium. It is then above all in this portion of the skull one may expect to get ideas of the original structure of the vertebrate neurocranium.

PARACHORDALS AND BASIOTIC LAMINAS

The *parachordals,* first described by Huxley in 1874, are traceable from cyclostomes to Man as paired cartilages flanking the notochord (DE BEER 1937). In *Selachii* (SEWERTZOFF 1897, 1899; VAN WIJHE 1904 a, 1922; DE BEER 1914 a, b, 1926, 1927; HOLMGREN 1940, 1943; and others) the parachordals are always formed in close contact with the notochord, constituting a shelf-like cord jutting out on either side (Fig. 40). In *Holostei* and *Teleostei,* however, the parachordals are described as cartilaginous rods frontally separated from the notochord by a broad gap (PEHRSON 1922; DE BEER 1937; and others). Caudally the rods converge and reach the notochord in the posterior part of the otic region. In actinopterygians the lateral margin of the parachordals are usually said to be directly attached to the basicapsular commissures. In selachians on the contrary these commissures are considered to be fused with the *basiotic laminas,* "a lateral extension from the parachordals underneath the otic vesicles" (VAN WIJHE 1922). However, as was found by HOLMGREN (1943) this difference between actinopterygians and selachians seems not to be generally acceptable (cf. p. 260). The present investigation has shown the same.

Already in 1882 STÖHR found that the *parachordals* in *Salmo salar* and *S. trutta* are not continuous rods from the very beginning of their morphogenesis; they instead chondrify from two separate centers, one "anterior parachordal plate" in the facial region remote from the notochord, and one "posterior parachordal plate" in the vagal region close to the notochord. Before the two chondral parts fuse, however, the posterior one was said to divide into two portions, charac-

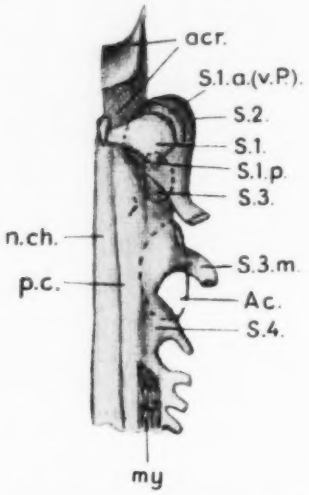

Fig. 40. *Squalus acanthias,* 12 mm. Reconstruction of entomesoderm, ventral view. From Holmgren (1940, Fig. 22).

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terized by different thickness: a thick anterior or "mesotic" portion extending to the middle of the sacculus, and a posterior or "occipital" portion fused to the occipital arch.

Also Sewertzoff (1895), Winslow (1898), Agar (1906), Swinnerton (1902), Pehrson (1922), Besrukow (1928) and Bhargava (1958) have suggested that the parachordals in *Acipenser, Protopterus, Lepidosiren, Gasterosteus, Amia, Esox* and *Mastacembulus,* respectively, chondrify from an anterior and a posterior center. Most authors, however, gave only a summary description. It is mentioned that in *Acipenser, Gasterosteus, Esox** and *Mastacembulus* the anterior and posterior parts chondrify independently. Therefore these findings conform with those of Stöhr in *Salmo.* In the two dipnoans mentioned the anterior and posterior parts are said to form separately but later fuse. Pehrson gave the most detailed description of the ontogeny of the parachordals. He then considered the anterior and posterior chondrification centres in *Amia* to be homologous with the "anterior parachordal plate" and the "mesotic part of the posterior parachordal plate", respectively, in *Salmo.* Stöhr's "occipital part of the posterior parachordal plate", on the contrary, was said not to be primarily present in *Amia* but gradually formed by caudad growth from the mesotic part.

Other authors (Tichomiroff 1885; de Beer 1927; Hubendick 1943) could not confirm these statements of the ontogeny of the parachordals in actinopterygians. De Beer for example considered the "anterior parachordals" to represent "polar

* Besrukow e.g. only described "eine frühere Anlage der vorderen Partie der Parachordalien im Vergleich zu deren hinteren Partie, die sich hinter den Weerzehr des Nervus vagus lagert" and then gave these different parts different letterings in his illustrations.

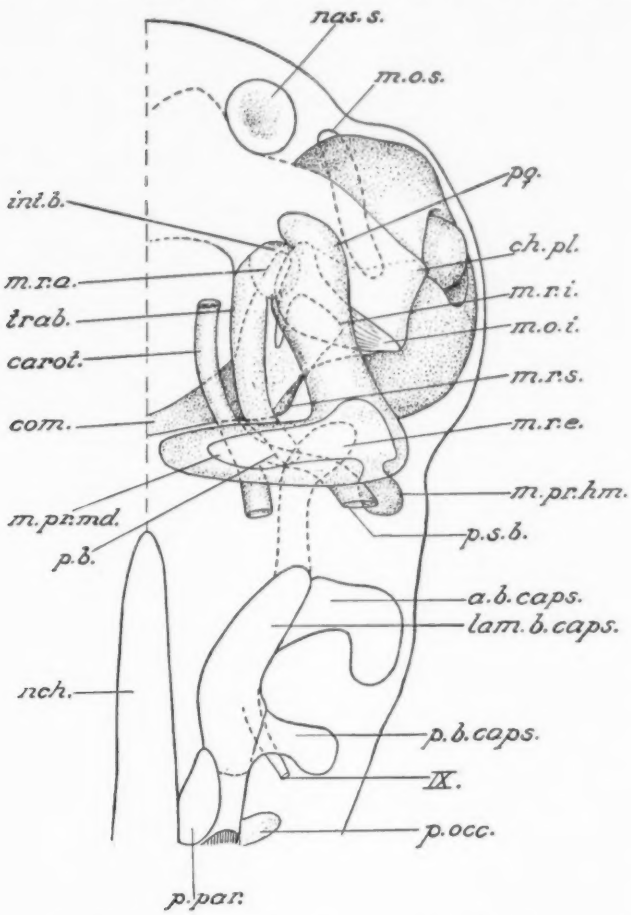

Fig. 41. *Salmo salar,* 55 days 6 hours, 4° C. Reconstruction of skull, ventral aspect. From HOLMGREN (1943, Fig. 14).

cartilages fused on to the anterior ends of the true parachordals". This suggestion, however, seems not to be founded on observations on early embryonic teleostome material.

An element completely new to the teleostomes was brought into the discussion when VAN WIJHE (1922), studying the ontogeny of *Squalus acanthias,* briefly suggested that the "anterior chondrification centres" described by PEHRSON in *Amia* —by HOLMGREN in short called the anterior parachordals, cf. p. 212—correspond to the *basiotic laminas* in *Squalus acanthias:* "Embryonen von *Amia* zeigen nach PEHRSON (1922, Fig. 2 und 4) die intressante Abweichung, dass nicht der eigentliche Körper der Parachordale, welcher der Chorda anliegt, sondern der

laterale Flügel, die Lamina basiotica, zuerst auftritt." The reason for this "revolutionary" homologization hence seemed only to be the fact that the anterior parachordals in *Amia* extend laterad from the mesotic parachordals close to the notochord. It may seem to be a too optimistic hypothesis, but none the less it has later been supported histologically and morphologically by HOLMGREN's studies of *Amia* and *Salmo* (1943) and by the present investigation on *Characidae* and other recent actinopterygians (cf. below).

HAMMARBERG (1937) adopted the hypothesis of VAN WIJHE and suggested that the anterior parachordals in *Lepisosteus,* which also diverge laterad from the notochord, are homologous with the lamina basioticas in *Squalus.* However, the circumstance that the anterior parachordals are not called lamina basioticas in his illustrations, seems to indicate some uncertainty on his part.

In 1940 HOLMGREN in a thorough study of early stages of several selachians found that the basiotic laminas (Fig. 40, l.bas.) of these fishes are certainly medially connected with the parachordals (pch.) but also that they are not at all derived from the parachordals, as SEWERTZOFF (1899) and VAN WIJHE (1904) had suggested. The basiotic laminas are instead formed *separately* as blastemal cords at the medioventral margin of the otic vesicles. They then chondrify in connection with the parachordals but are still histologically and morphologically independent.

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The selachian parachordals on the other hand are formed in connection with the notochord (ch) and extend as far as to the tip of the notochord. Here they are, according to HOLMGREN, not directly but via the accrochordal tissue connected with the polar cartilages (p.b.). Rostrally the basiotic laminas play a more important part in the formation of the basis cranii than the parachordals do, especially in the *Batoidei;* this portion of the laminas is usually called the basiotic plate.

When considering the *origin* of the basiotic laminas and the parachordals HOLMGREN showed that both are formed from the hyal and the first branchial (metotic) somites (s. 3, 4). Concerning the 7—8 mm stage of *Squalus acanthias,* HOLMGREN writes: "The first branchial somite is developed as the hyoid somite with a parachordal portion, a basiotic part and *a muscular process to its branchial arch.** The lamina basiotica thus consists of a lamina basiotica anterior and posterior, respectively. The second branchial somite differs from the foregoing as there is a myotome belonging to it. The myotome part is developed in a portion of the somite corresponding perfectly to the basiotic laminas belonging to the

* Italics by the present author. The fact that the visceral muscle primordia of at least the two anterior selachian branchial arches are initially continuous with the respective branchial (metotic) somite was earlier claimed by e.g. MARSHALL (1881), VAN WIJHE (1922) and GOODRICH (1932). Certainly, no segmentation of the mesoderm above the anterior branchial arches (the metotic mesoderm) is described in teleostomes and dipnoans, but in analogy with the conditions in selachians just only the connection between the anterior branchial arches and the metotic mesoderm or endomesenchyme above them might indicate the position of the two original anterior branchial (metotic) somites in these fishes. Cf. below.

first branchial and hyoid somites respectively. *The difference is that in the hyoid and first branchial somites also the myotome portion transforms into skeletal tissue.*"*

HOLMGREN (1943) also investigated *Amia* and *Salmo*. He then found that even in these fishes basiotic laminas are formed at early stages. Regarding *Amia* this discovery was a more important contribution to the understanding of the morphology of the basal parts of the teleostomian skull than the hypothesis of VAN WIJHE, made on the same species (cf. above).

In the details, however, objections can be raised against HOLMGREN's statements. HOLMGREN found at the 8 mm stage of *Amia* that "the parachordal except for its most caudal portion is still quite blastematic and forms a thickish band on the side of the notochord. In front of the anterior basicapsular commissure this band becomes thinner, less differentiated, forming a mesenchymatic membrane between the basiotic lamina + the polar cartilage and the notochord". He then generalizes, however: "From this description it is clear that, as already was suggested by VAN WIJHE (1922), what earlier workers described as parachordals is the basiotic lamina." In fact VAN WIJHE only considered the *anterior* parachordals to be the basiotic laminas** (cf. p. 257). For that reason HOLMGREN should have confined himself to suggest that what earlier workers described as the *anterior* parachordals correspond at least in part to the basiotic lamina. That is of importance in this connection.

As to *Salmo,* HOLMGREN's investigations of 1943 showed that *Amia* and *Salmo* are rather alike in this respect (Figs. 41, 42 A). A "posterior parachordal" (p.pch.) formes in the postvagal region, lying close to the notochord (nch) and in *Salmo* (Fig. 41) "reaching (frontally) a separate prochondrial rod (l.bas.), running frontad at the medioventral border of the auditory vesicle. This rod is apparently STÖHR's anterior parachordal plate.*** If compared with Selachians, however, the rod must be considered to be the basiotic plate which has the same relation to the auditory vesicle and which develops laterally to the true parachordals which are there present. In very young embryos of *Salmo salar* the space between the notochord and this basiotic plate is filled in with an undifferentiated mesenchyme with scattered cells" (HOLMGREN 1943). This mesenchyme (the "true" or anterior parachordals is described also in *Amia* (cf. Fig. 42 A). Its future fate in *Amia* is not mentioned, however, (cf. p. 262) whereas in *Salmo* it gradually chondrifies and diminishes to a narrow strip between the notochord and the basiotic lamina. Finally, it becomes posteriorly "completely fused with the basiotic lamina and is no longer distinguishable", whereas the anterior portion chondrifies as "the dorsal

* Italics by the present author.

** HOLMGREN's "posterior parachordal" (Fig. 42 A, p.par.) is situated close to the notochord and on a level with the posterior portion of the otic vesicle and with the preoccipital arch (erronously called the pila occipitalia in his Fig., cf. PEHRSON 1922, Figs. 5—7) and the first myomere. PEHRSON had, however, already described (1922, p. 5—10) a separate "posterior chondrification" of the parachordal in this region (cf. p. 257).

*** Cf. Amia above.

surface of the rudiment of the prootic bridge. Thus the floor of the brain-case of the chordal part of the skull is practically made up of the laminae basiotici and the anterior (prootic bridge) and posterior (occipital) parts of the parachordals. In the course of development the anterior end of the border of the basiotic lamina extends as a process frontad to meet the caudal end of the trabecular system (the polar cartilages). This part seems to be comparable with the preotic part of the basiotic lamina in Selachians (HOLMGREN 1940)" (HOLMGREN 1943).

HUBENDICK (1943) did not find any basiotic laminas in *Leuciscus rutilus* but considered that the parachordals partly might represent such elements. In other teleosts basiotic laminas are not described until now. *Polypterus* is also unknown in this respect because of the lack of early embryonic stages. As to chondrosteans and holosteans cf. above. Not even in the different investigations of *Dipnoi* (cf. p. 269) anything is mentioned of any basiotic laminas.

Of the cyclostomes separately formed anterior and posterior parachordals are described in *Petromyzon* (SEWERTZOFF 1916, JOHNELS 1948), whereas basiotic laminas have not been discerned. *Myxine* (HOLMGREN 1946), probably also *Bdellostoma*, have, however, basiotic laminas as well.

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As there are no investigations of other teleosts supporting HOLMGREN's theory concerning the formation of parachordals and basiotic laminas, the studies of the characids may have a certain interest.

As was shown in detail in chapter III both parachordals and basiotic laminas do form also in *Hepsetus*, the parachordals being situated close to the notochord and the basiotic laminas ventromedially to the otic vesicles. From the first stage the two elements are histologically and usually also morphologically discerned from each other, as the basiotic laminas have more concentrically arranged cells concentrated into a longitudinal cord. They are, however, each formed into three parts not one or two as HOLMGREN states for *Amia* and *Salmo*. Their ontogeny can be followed in Figs. 2, 8, 13, 16, 26, 34 (l.bas.a., m., o., pch.a., m., o.).

The anterior parachordals (Fig. 2, pch.a.) differentiate later than the other two parts of the parachordals. Initially they consist of a large but only sparsely nucleified blastema, situated in the facial region and connecting the entire lateral surface of the notochord (ch.) with the entire medial border of the anterior basiotic lamina (l.bas.a.). When chondrification sets in, the anterior parachordals narrow and are restricted to the dorsolateral margin of the notochord. Primarily they do not reach the tip of the notochord but fuse with each other above it (Fig. 13). Later they chondrify from behind into one common piece and separate from the mesotic parachordals (Fig. 26). This narrow strip of the anterior parachordals finally fuses caudolaterally with the anterior basiotic laminas and is then no longer distinguishable (Fig. 34). Rostrally, however, the anterior parachordals can still be histologically discerned as the prootic bridge (po.b). This bridge constitutes the roof of the posterior myodome (p.my.), left behind when the notochord regresses (Fig. 35).

The forming of the anterior parachordals in *Hepsetus* hence conforms with that in *Amia* and *Salmo* as described by HOLMGREN (1943) (cf. above). However, if compared to the remaining parts of the parachordals, it seems to take place earlier in *Hepsetus*. In *Amia* HOLMGREN did not mention the fate of the blastemal anterior parachordals (cf. p. 259). An investigation of his material kept at this Institute) shows, however, that they will chondrify only posteriorly, contrary to what happens in *Salmo* and *Hepsetus*.

Considering STÖHR's "anterior parachordal plates" in *Salmo* they are said to be formed at the anteromedial border of the otic vesicles and at a distance from the notochord; they later fuse with the posterior end of the "trabecles" (polar cartilages). However, judging from this description they should instead correspond to the anterior *basiotic laminas* in *Hepsetus* (cf. below), not to the anterior parachordals. This theory seems also to apply to the anterior part of the "parachordals" in *Protopterus, Lepidosiren, Acipenser, Amia, Esox, Gasterosteus* and *Mastacembulus* (cf. p. 258).

The mesotic (pch.m.) and occipital parachordals (pch.o.) in *Hepsetus* differentiate earlier than the anterior ones and at all stages connect with the entire lateral side of the notochord. As is seen from Fig. 2 they are formed in the glossopharyngeal and vagal regions, respectively. The mesotic parachordals are dorsally connected with the preoccipital arches (pr. o.) and laterally with the mesotic laminas (l.bas.m.), and the occipital parachordals with the occipital arches (p.o.) and the occipital basiotic laminas (l.bas.o.), respectively. Like the neural arches in the trunk somites (cf. e.g. GOODRICH 1918, 1930) the occipital and preoccipital arches form caudally to their dorsal nerve roots—the glossopharyngeal and vagal nerve root, respectively—and extend upwards laterally to the nerve cord (the brain). Many authors (GOODRICH 1911, 1918, KERNAN 1915, DE BEER 1931, VAN SETERS 1922 and others) have earlier claimed that these arches have a vertebral origin. This theory seems acceptable.

The mesotic and occipital parachordals have fused chondrally at the 8 mm stage but are histologically delimited (Fig. 16). At the 12.6 mm stage (Fig. 26) the mesotic parachordals reduce rostrally and give place to the ductus communicans transversus of the sacculi (cf. p. 242). The occipital arches grow rostrad on to the labyrinths (Figs. 6, 15) and fuse (1) with the anterior otic cartilages (ca.a.) rostrally, forming a tectum synoticum above the hind brain (Fig. 25, t.o.), (2) with the posterior otic cartilages (ca.p.), laterally, constituting the cartilagenous lateral wall of the posterior semicircular canals and (3) with the preoccipital arches, ventrally, which form the ventral portion of the posteromedial wall of the posterior semicircular canals as well as of the lagenas.

According to their position the mesotic and occipital parachordals together seem to represent the "posterior parachordals" in *Amia* and *Salmo* as described by HOLMGREN (1943).

STÖHR's division of "the posterior parachordal plate" in *Salmo* into two pieces,

an anterior "mesotic" and a posterior "occipital", is founded only on their morphological appearance (cf. p. 257) and has not been verified, illustrated or commented on by HOLMGREN. The "occipital" portion, however, being formed in connection with the occipital arch, obviously represents HOLMGREN's "posterior parachordal", situated in the same position. For that reason it also seems to correspond to the occipital parachordal in *Hepsetus*.—STÖHR's "mesotic" portion is, on the other hand, more dubious. According to its supposed formation from the "posterior parachordal plate" it should represent the mesotic parachordal in *Hepsetus*. However, STÖHR says that the mesotic part constitutes the medial border of the otic capsule, furthermore that it is laterally connected with the posterior basicapsular commissure and finally that it reaches to the middle of the sacculus. Accordingly it seems more reasonable that STÖHR's "mesotic parachordal" in *Salmo* is represented by the mesotic *basiotic lamina* in *Hepsetus*. This also tallies with the reconstruction of about the same stage of *Salmo salar* published by HOLMGREN (Fig. 41) and with the morphology of the skull base in *Gasterosteus*, *Esox* and *Mastacembulus* (p. 258). In them the "posterior parachordals" have chondrified only at the base of the occipital arches and have no such extension rostrad as reported by STÖHR in *Salmo*.

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As to the *basiotic laminas* in *Hepsetus* it has been found that they also only partly tally with those of *Amia* and *Salmo* as HOLMGREN (1943) described them. They certainly form under the medial rim of the otic vesicles and are larger and differentiate earlier than the parachordals. However, each of them is not formed in one piece* but—like the parachordals in *Hepsetus*—primarily into three parts: (1) an anterior part (Fig. 2, l.bas.a.) in the facial region, situated at the anteromedial margin of the otic vesicle and connected medially with the anterior parachordal, laterally with the anterior basicapsular commissure (a.bc.c.) and ventrolaterally with the muscle rudiment (the mesoderm)** of the hyal arch (s. 3 m.); (2) a mesotic part (l.bas.m.) in the glossopharyngeal region, situated at the posteromedial rim of the otic vesicle and connected medially with the mesotic parachordal, laterally with the basivestibular commissure (bv.c.) and ventrolaterally with the muscle primordium of the first branchial arch (s. 4 m); and (3) an occipital (posterior) part (l.bas.o.) in the vagal region, primarily sending out a rostrolateral process towards the posteroventral rim of the otic vesicle (Fig. 2) and connected medially with the occipital parachordal, laterally (from the third stage, Fig. 13) with the posterior basicapsular commissure (p.bc.c.) and ventrolaterally with the muscle rudiment of the second branchial arch (Fig. 2, s. 5 m.). When chondrifying the cells arrange themselves differently in the two anterior parts and also concentrate so that a morphological border is formed between them (Figs. 16, 17, 26). The occipital basiotic laminas chondrify from the mesotic laminas and have no anterior demarcation after their

* Cf., however, p. 266 and HOLMGREN's reconstruction, Fig. 41.
** Cf. p. 260, note.

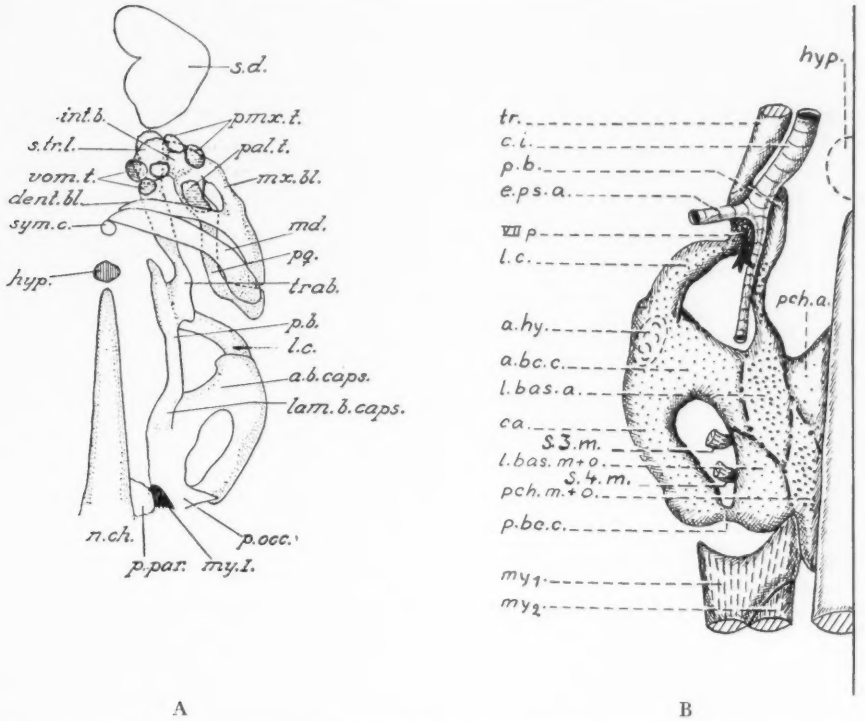

Fig. 42. *Amia calva,* 8 mm. Reconstruction of skull, ventral aspect. A, from HOLMGREN (1943, Fig. 29); B, the shape of skull if also the anterior part of the parachordals is accounted for. The relations to internal carotid, palatine nerve and muscle rudiments of the first two branchial arches are shown. Indications as in Figs. 1—2, 13. 62 ×.

chondrification. The anterior basiotic laminas extend back along the notochord and form posteriorly the ventromedial covering of the sacculi. The mesotic basiotic laminas on the other hand protect the ventrolateral border of the sacculi and together with the occipital laminas also the ventrolateral border of the lagenas.

The anterior basicapsular and basivestibular commissures, which were blastemal during the first stage, gently merge into the anterior and posterior otic cartilages, respectively (Fig. 2). Medially the flat commissures with their cells stacked on to each other are, on the contrary, delimited from the cord-like basiotic laminas, characterized by their more rounded and concentrically arranged cells. As, furthermore, the posterior basicapsular commissures at the two following stages differentiate from the posterior otic cartilages and join the occipital basiotic laminas behind the glossopharyngeal nerve (Figs. 8, 13), there is consequently every reason to suggest that also the other two commissures originally have extended from the otic cartilages. In early characids or their predecessors the otic cartilages might then have been formed independently, as they are in *Acipenseridae*

(the posterior otic cartilages) (SEWERTZOFF 1928) and *Selachii* (VAN WIJHE 1904 a, 1922; and others).

Considering the basiotic laminas comparisons can only be made with *Salmo* and *Amia* as described by HOLMGREN (1943), because they are not reported in any other teleostomian. The similarities between these genera have already been pointed out earlier (p. 261) and are verified by the present author. HOLMGREN does not describe any division of the basiotic laminas in *Salmo,* but according to their morphology and position only along the medioventral rim of the otic vesicles (Fig. 41) they seem to correspond to the anterior and mesotic basiotic laminas in *Hepsetus.* HOLMGREN has also illustrated (although not described) a posterior portion of the laminas, extending back to the occipital arches (Fig. 41) and situated laterally to the "posterior parachordals". These posterior laminas are obviously homologous with the occipital basiotic laminas in *Hepsetus.*

As to *Amia* HOLMGREN in his chapter "The relations of the palatoquadrate and the trabecula" only gives a general description of the basiotic laminas (p. 261, cf. Fig. 42). In order to get a more detailed comparison with *Hepsetus* the author has made a more thorough investigation on the same material (Fig. 42 B). It shows that each basiotic lamina form in two parts: (1) an anterior, earlier differentiated portion in the facial region (l.bas.a.) (= PEHRSON's "anterior chondrification", p. 258), which has exactly the same position to the otic vesicle, polar cartilage and so on as the anterior basiotic lamina has in *Hepsetus,* and therefore they probably are homologous, (2) a posterior part (l.bas.m.+o.) in the glossopharyngeal and vagal regions (not described by PEHRSON but partially illustrated in his Fig. 4) which according to its position and relations to the muscle rudiments of the first (s. 4 m) and second branchial arches seem to correspond to the mesotic and occipital basiotic laminas in *Hepsetus.* This posterior part soon chondrifies in connection with the posterior parachordal (9—11 mm) and is then no longer discernible as a separate element; the limit towards the posterior basicapsular commissure is, however, left.

The floor of the chordal part of the chondrocranium in *Hepsetus* is thus principally comparable to that of *Amia* and *Salmo,* though *Hepsetus* seems to be more primitive in having a further advanced and more distinct segmentation (cf. p. 268).

Considering the other characids investigated the embryos are too differentiated to admit any conclusion whether their parachordals and basiotic laminas are serially formed (segmented) or not.

Of the other teleosts available (p. 205--206) only *Esox, Heterotis* and *Perca* give any sign of an early double nature of the otic floor. *Heterotis* has mesenchymal basiotic laminas formed at the ventromedial margin of the otic vesicles before any sign of the parachordals can be seen. As in *Amia, Salmo* and *Hepsetus* they are ventrolaterally connected with the muscle primordia of the hyal and the first and second branchial arches. They seem to chondrify from two centers, one ante-

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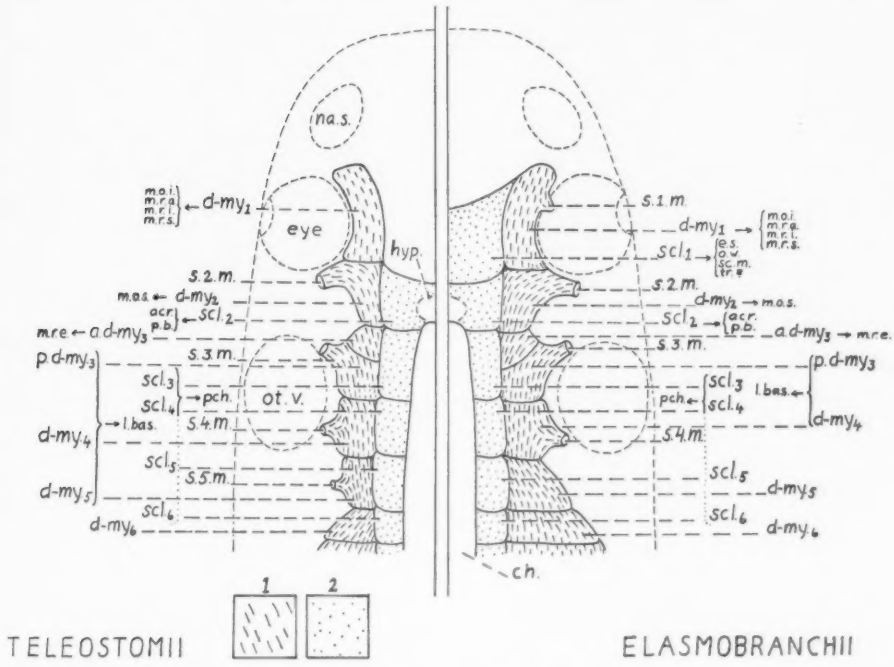

Fig. 43. Diagrammatic representation to show the segmentation of the skull in teleostomes (left) and elasmobranchs (right). 1, dermo-myotomes; 2, sclerotomes. The fate of these units is shown by letterings in the Fig.

rior in the facial region and one metotic in the glossopharyngeal region. The parachordals are simultaneously chondrified into one single piece, flanking the whole lateral border of the notochord even anteriorly.

In *Esox* the basiotic laminas constitute a direct continuation forwards of the real myotomes and are, as in *Heterotis* and *Perca,* formed before the slender parachordals appear. In neither *Esox* nor *Perca* any separate chondrification of the basiotic laminas and the parachordals has been observed. The early difference in the arrangement of their blastemal cells dissappears and the parachordals and the basiotic laminas can no more be distinguished from each other. The blastemal laminas seem, however, to form the essential part of the basis cranii, judging from their extension in the blastemal stage.

Consequently it might generally be said (1) that separate basiotic laminas and parachordals so far have been found in the blastemal stages of *Salmo, Characidae, Esox* and *Heterotis* (as to *Amia* cf. p. 226), (2) that the basiotic laminas are even after their chondrification to be discerned in *Salmo, Characidae* and *Heterotis,* (3) that the basiotic laminas in these fishes morphologically dominate over the parachordals and (4) that traces of a serial formation of these elements

("mesenchyme somites", cf. below) have been found in *Hepsetus* and partially also in *Salmo* and *Heterotis*.

The *general conclusions* may be summarized as follows. (1) The serial formation of the parachordals and basiotic laminas in *Hepsetus* and also partially in *Amia, Salmo* and *Heterotis*, their regular relations to the dorsal nerve roots*, as well as the fact that the mesoderm of the hyal arch and the first and second branchial arches (cf. p. 260, note) is primarily connected with the anterior, mesotic and occipital basiotic laminas, respectively, indicate that *also in higher teleostomes*—as in selachians, cf. p. 260—there may exist traces of an *original segmentation* of the metotic region of the head. This segmentation, however, applies only to the endomesenchyme ("mesenchyme somites"), not to the mesoderm. (2) The disappearance of the mesodermal segmentation of the metotic region (and most of the prootic, cf. p. 289) in higher teleostomes may be due to a form of *shortening* (abbreviation) of the ontogeny (cf. STARCK 1955; and others) of parachordals and basiotic laminas. (3) If this theory is correct the anterior parachordals and basiotic laminas in higher teleostomes may represent the 3rd (hyal) somites (Fig. 43), the mesotic parachordals and basiotic laminas the 4th (1st branchial or metotic) somites and the occipital parachordals and the basiotic laminas the 5th (2nd branchial or metotic) somites. (4) If compared with their trunk and with the selachians (Fig. 43), and supposing that the occipital and preoccipital arches really represent neural arches (p. 263), it seems reasonable to assume each of the parachordal portions (formed of endomesenchyme and situated close to the notochord) to represent a *sclerotome* (cf. p. 269). As to the basiotic laminas it is for the same reasons and according to their position laterally to the parachordal portions (sclerotomes), tempting to consider them as transformed *dermo-myotomes***. In order to get a closer understanding of this problem it is, however, necessary to investigate also lower actinopterygians, dipnoans and selachians.

In no investigations of lower teleostomes and dipnoans any basiotic laminas (cf. p. 261) are hithertoo described. To get a thorough information and idea of these elements the present author has investigated *Acipenser, Polypterus* and *Dipnoi*. The study has revealed that also in these fish groups a distinction between parachordals and basiotic laminas can be made.

A large material of *Acipenser* has been studied (cf. p. 205) and some reconstructions have been made to illustrate the ontogeny (Figs. 45—46). All *Acipenser* species investigated are primarily alike in this respect. In late neurula stage of *A.ruthenus* (where the otic vesicles are still thick placodes) the mesoderm in the

* As is well-known, the dorsal nerve roots of the prootic segments in *Selachii* (which are best investigated in this connection) are the opthalmicus profundus, the trigeminal and the facial; those of the metotic segments are the glossopharyngeal and the compound vagus, one branch of which corresponds to each somite in this region (VAN WIJHE 1882, GOODRICH 1918, DE BEER 1922).

** As in *Amia* and *Lepisosteus* (SCHREINER 1902) and *Salmo* (WILLCOX 1899) the first true (occipital) myotome in *Hepsetus* is formed in the 6th somite.

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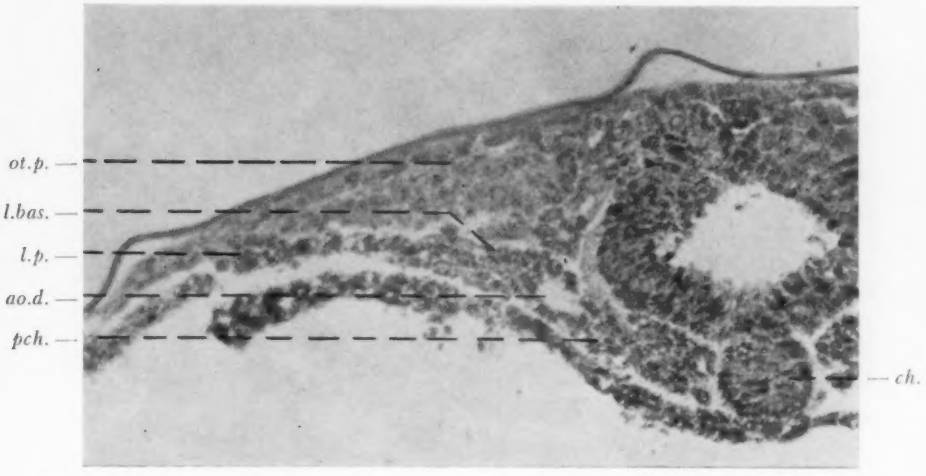

Fig. 44. *Acipenser ruthenus*, late neurula stage. Transverse section to show the position of parachordal and basiotic laminar rudiments. 225 ×.

otic region is divided by the dorsal aorta (Fig. 44, ao.d.) into a medial sclerotomal part close to the notochord (the prospective parachordals /pch./), and a lateral part close to the ventromedial margin of the otic placode (ot. p.), the prospective basiotic lamina (l.bas.). The latter is ventrolaterally continuous with the lateral plate mesoderm (l.p.). No segmentation of the mesoderm of the otic region can be seen.

In e.g. *A. ruthenus* 8.6 mm (Fig. 45 A) the parachordal (sclerotomal) and basiotic laminar part of the mesoderm has broken up into endomesenchyme; the lateral plate is split by the visceral clefts, and visceral arches are forming. There is, however, still a loose connection left between the lamina basiotica and the lateral plate mesoderm of the hyal and first and second branchial arches (s. 3—5 m). At these places (l.bas.a., l.bas.m., l.bas.o.) the lamina is thicker and in that way segmented in a way comparable to that in *Hepsetus* (Figs. 2, 8).—In the orbito-temporal region three prootic somites are formed, each with a head cavity (Figs. 45 A, 68).

In the facial region the basiotic lamina is, on each side, laterally connected with the anterior basicapsular commissure (a.bc.c.) of the anterior otic cartilage (ca.a.) and medially with the parachordal (pch.). Except for another connection caudally (in the vagal region) the lamina basioticas and the parachordals are free from each other. Thus a large foramen is formed between them, in which the acustico-facial ganglion is situated. Like the parachordals and the anterior otic cartilages the basiotic laminas consist of large endomesenchymal cells which are laden with yolk. They are, however, grouped into a thick cord that can be morphologically delimited from the other skeletal elements mentioned. It is finally of special

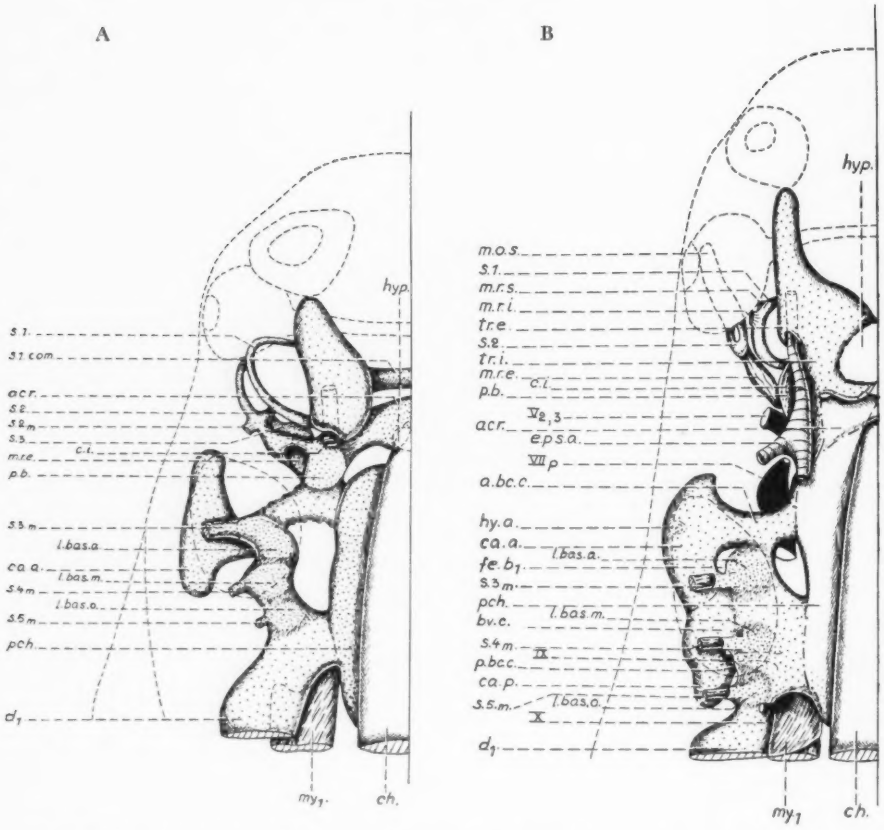

Fig. 45. Reconstruction of left part of neural chondrocranium, ventral view. A, *Acipenser ruthenus* 8.6 mm; B. *A.stellatus* 10 mm. Indications as in Figs. 1—2, 13. 100 ×.

interest for the understanding of the nature of the basiotic laminas that they are caudolaterally joined to the dermatomic mesenchyme of the first occipital somites (d_1)*, the mesenchyme of which so far has the same histological appearance as that of the laminas. The first permanent myomeres (my_1) lie between the posterior end of the parachordals and the basiotic laminas.

The caudal junction between the blastemal basiotic laminas and the first occipital dermatomes still remains in a 10 mm *A. stellatus* (Fig. 45 B). The demarcation towards the parachordals is, on the contrary, even more distinct, as the parachordals are beginning to chondrify, whereas the laminas are still blastemal. Another histological difference is the fact that the cells of the basiotic laminas have lost their yolk, while those of the parachordals have not. The connection between the

* Probably the sixth somite; the segmental number is, however, difficult to settle without knowing the number of the occipital ventral nerve-roots (cf. DE BEER 1937).

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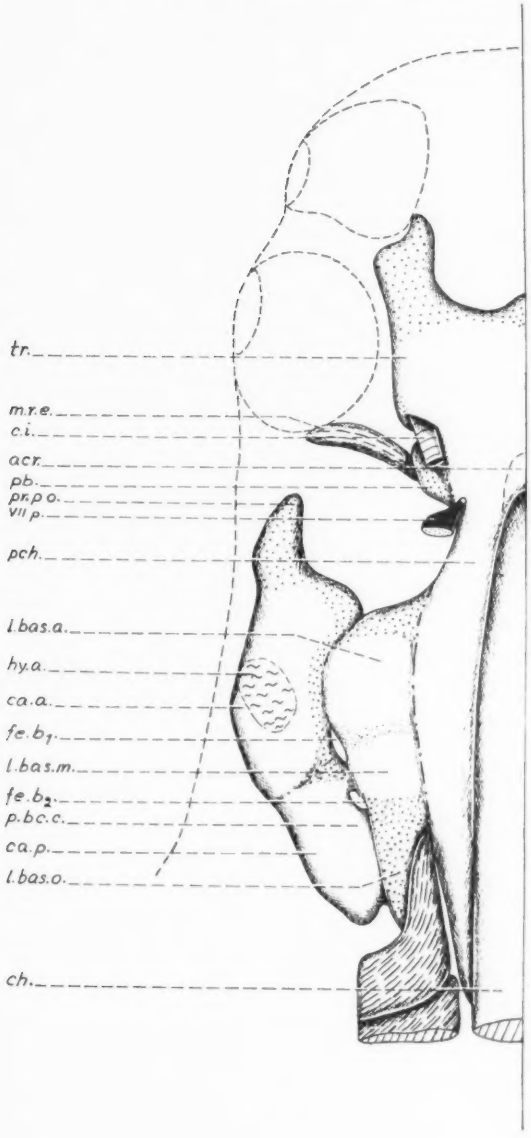

Fig. 46. *Acipenser stellatus*, 13 mm. Reconstruction of left part of neural chondrocranium. Indications as in Figs. 1—2, 13. 100 ×.

parachordals and laminas has broadened, but a foramen is still left in the centre. Further, the laminas are laterally connected with the flat basivestibular (bv.c.) and posterior basicapsular commissures (p.bc.c.) of the still sparsely nuclified posterior otic cartilages (ca.p.). For that reason this stage principally resembles the third stage of *Hepsetus* (p. 224, Fig. 13). Finally is to be mentioned that the

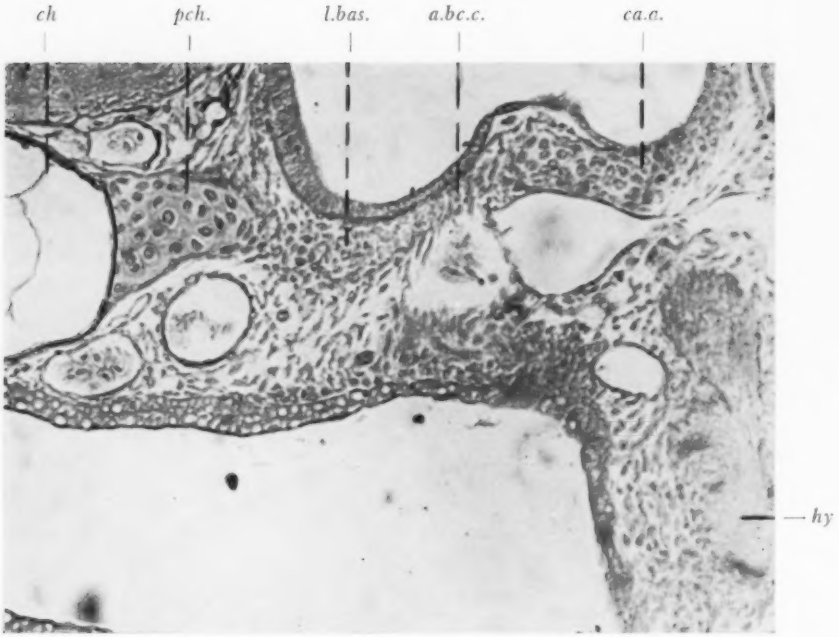

Fig. 47. *Acipenser stellatus*, 13 mm. Transverse section through right body wall, on a level with the posterior end of the hyal arch. The section shows the position of the parachordal, basiotic lamina and otic cartilage. 160 ×.

muscle primordia of the hyal and the first two branchial arches (Fig. 45 B, s. 3—5 m.) are now differentiated into muscle tissue. There are, however, still loose blastemal connections left with the basiotic laminas, and here the laminas are somewhat thicker as they were before (l.bas.a., m., o.).

At the 13 mm stage of *A.stellatus* (Fig. 46) the parachordals have chondrified and are now connected along the entire length with the basiotic laminas. The latters are anteriorly and posteriorly still blastemal but are chondrifying towards the center. Their connection with the first occipital dermatomes is dissolving. There is still a distinct morphological and histological demarcation between the basiotic laminas and the chondral elements formed near them (the parachordals and the anterior and posterior otic cartilages). Their chondrocytes are arranged differently and those of the demarcation zone are smaller (Fig. 47); the sparsely developed intercellular substance of the demarcation zone contains many fibres.

To conclude: the basiotic laminas in *Acipenser* are formed from that part of the unsegmented mesoderm of the otic region, which is situated at the ventromedial margin of the otic placodes (vesicles). They are continuous with the mesoderm of the hyal and first and second branchial arches. At these places they are somewhat thicker and therefore divided in a way comparable to that in

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Hepsetus ("mesenchyme somites"); the parachordals are undivided. Caudally the basiotic laminas are, at the blastema stage, joined to the dermo-myotomes of the first permanent (occipital) somites. The further differentiation occurs later than, and partially apart from, the parachordals (sclerotomes) located along the notochord. The demarcation lines between them and the commissures from the otic cartilages remain also at the late chondral stage.

The ontogeny of the chondrocranium of *Polypterus* is incompletely known. A few specimens of *Polypterus senegalus* have been described by WINSLOW (1898) and by BUDGETT (1902) and GRAHAM KERR (1907) (6.75, 8, 9.3, 24, 30 mm); these specimens were later reinvestigated by MOY-THOMAS (1933) and have also been examined by the present author. For comparisons a 9.6 mm specimen of *P.senegalus* (?) (JOHNELS 1954), captured by the second Swedish Gambia expedition, has been studied.

At the 6.75 mm stage (sagittally sectioned and rather badly preserved) no distinct basiotic laminas have been found. The parachordals are situated along the notochord as are earlier described and reconstructed by MOY-THOMAS. In the 8, 9.3 and 9.6 mm specimens the essential parts of the chondrocranium are chondrified (cf. MOY-THOMAS and Fig. 48). In spite of this the basiotic laminas (l.bas.) can be discerned in all three specimens for the same reasons as at comparable stage of other actinopterygians (p. 272). The parachordals are, as the basiotic laminas, formed in one piece; the specimens are too advanced to have any ventrolateral connection with the muscle rudiments of the hyal and two anterior branchial arches. The basiotic laminas are rather short and do not yet reach the tip of the notochord (ch.), rostrally, or the occipital arches (p.o.), caudally. The otic cartilages (ca.) are—as in characids and acipenserids—medially connected with the anterior and posterior basicapsular (a.bc.c., p.bc.c) as well as with the basivestibular commissures (bv.c.). The anterior and posterior basicapsular foramina are already at this stage very small (fe.b.$_{1,2}$), and as usual they are situated in the demarcation line between the basiotic laminas and the otic cartilages.

In short has been found that in *Polypterus* like in other actinopterygians and also in selachians (cf. above) the basiotic laminas constitute an important part of the otic region. As to their origin, however, the available polypterids cannot furnish any information.

Among the *Dipnoi Neoceratodus* has been thoroughly studied by GREIL (1913). He did not, however, describe any basiotic laminas, although he obviously observed them: his Figs. 426—428 show a separate chondral cord at the ventromedial margin of the labyrinths.

An extensive material of *Neoceratodus* has been investigated by the present author (cf. p. 205). It reveals that actually basiotic laminas are formed also in this fish. It has also been found that the otic mesoderm is unsegmented. As in

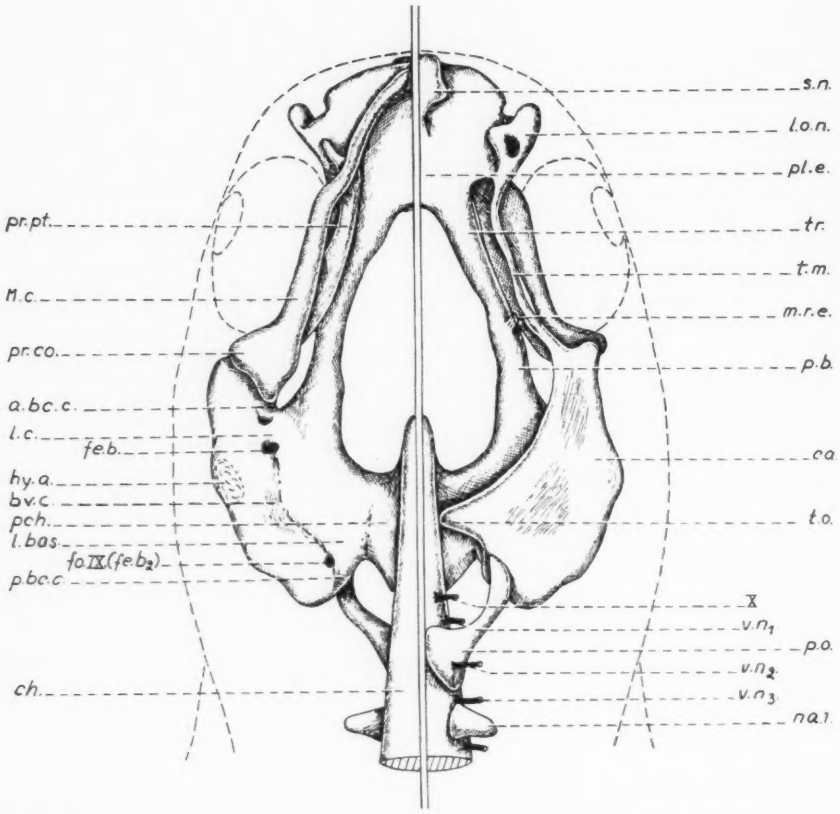

Fig. 48. *Polypterus senegalus*, 9.6 mm. Reconstruction of neural chondrocranium and mandibular arches. Left ventral view, right dorsal view. Indications as in Fig. 33. 60 ×.

Acipenser the first permanent (occipital) myomeres* in early embryonic stages of *Neoceratodus* (e.g. 8.6 mm, Fig. 49) are situated between the caudal part of the parachordals and the basiotic laminas, and as in *Acipenser* the laminas form a direct anterior continuation of the mesenchymal first occipital dermatomes (d_1). Anteriorly the rudimentary basiotic laminas are continuous with the external rectus rudiments (m.r.e.) (see below p. 280), which, as the basiotic laminas and the parachordals (pch.), still consist of indifferent yolk-laden endomesenchyme.** Medially the basiotic laminas are connected with the parachordals but yet dis-

* The first ventral spinal nerve innervates the second myomere. According to SEWERTZOFF (1902) and KRAWETZ (1910) this myomere belongs to the sixth segment, a statement in agreement with the facts concerning the segmentation of the head skeleton found by the present author.

** As to the histological differences between endo- and ectomesenchyme in *Dipnoi* compare p. 208.

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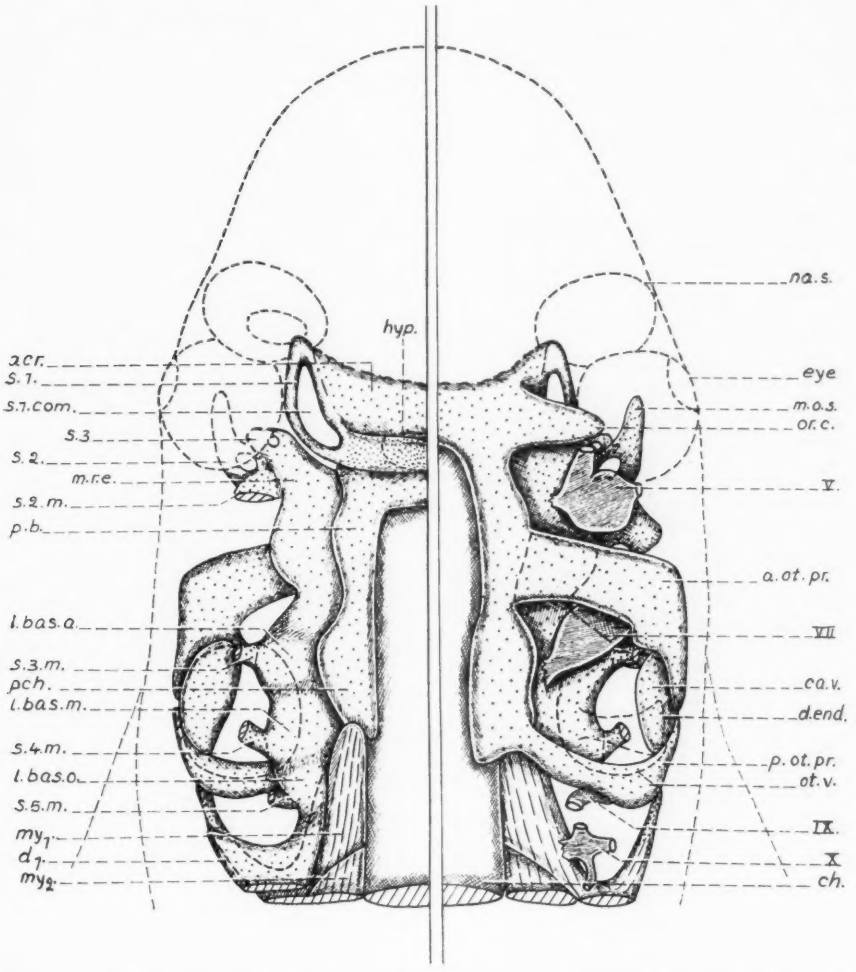

Fig. 49. *Neoceratodus forsteri,* 8.6 mm. Reconstruction of neural chondrocranium. Left, ventral view; right, dorsal view. Indications as in Figs. 1—2, 13. 75 ×.

cernible from them, as their densely arranged cells form a distinct cord. The parachordals are unsegmented and reach the tip of the notochord (ch.). Behind the hypophysis (hyp.) they are connected with each other below the notochord and have rostrally fused with the acrochordal tissue (acr.) (cf. below). Laterally the basiotic laminas have not yet reached the floor of the future otic cartilages (ca.v.).

During the further differentiation of the basiotic laminas their cells tend to agglomerate still more, whereas their connections with the muscle rudiments of the branchial arches and with the external rectus muscle rudiments instead

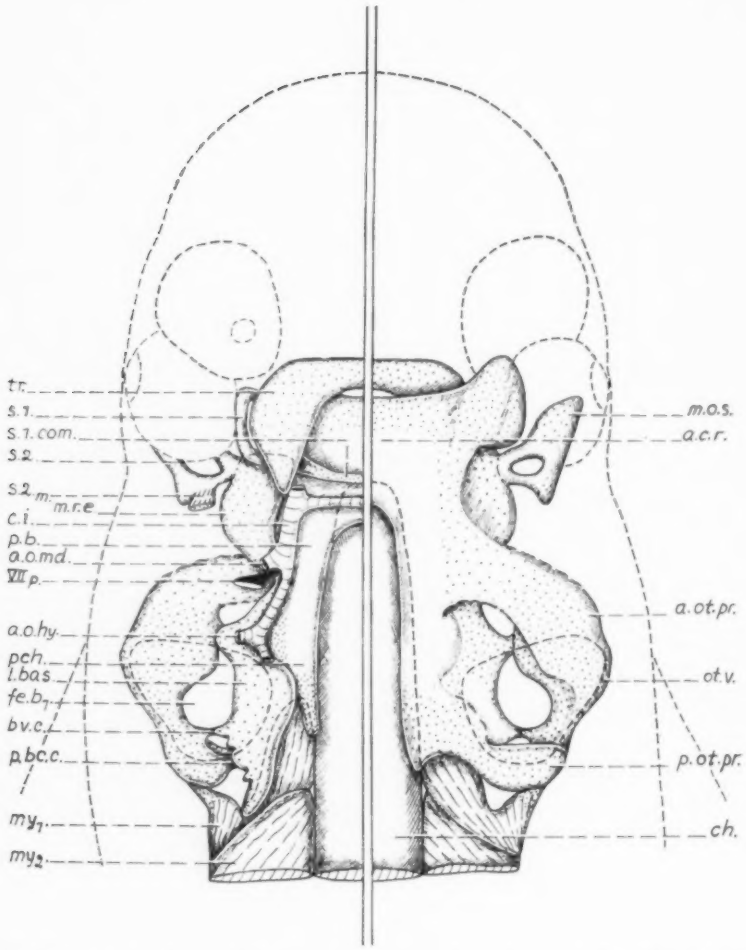

Fig. 50. *Neoceratodus forsteri*, 10.6 mm. Reconstruction of neural chondrocranium. Left, ventral view; right, dorsal view. The position of internal carotid and palatine nerve is shown as a comparison with actinopterygians. Indications as in Figs. 1—2, 13. 63 ×.

dissolve. This is shown at e. g. the 10.6 mm stage (Fig. 50). The caudal fusion of the basiotic laminas with the dermo-myotomes remains, however, comparatively long. Histologically they become better delimited from the parachordals, as these are chondrifying earlier (cf. *Acipenser*, p. 270).

The otic cartilages are definitely forming at the 10.6 mm stage. The earlier independent *ecto*mesenchymal floor (Fig. 49, ca.v.) has then fused with the *endo*mesenchymal processes (Figs. 49, 50, a., p.ot.pr.), rising from the parachordals at this and the preceeding stage and enveloping the otic vesicles from anterior

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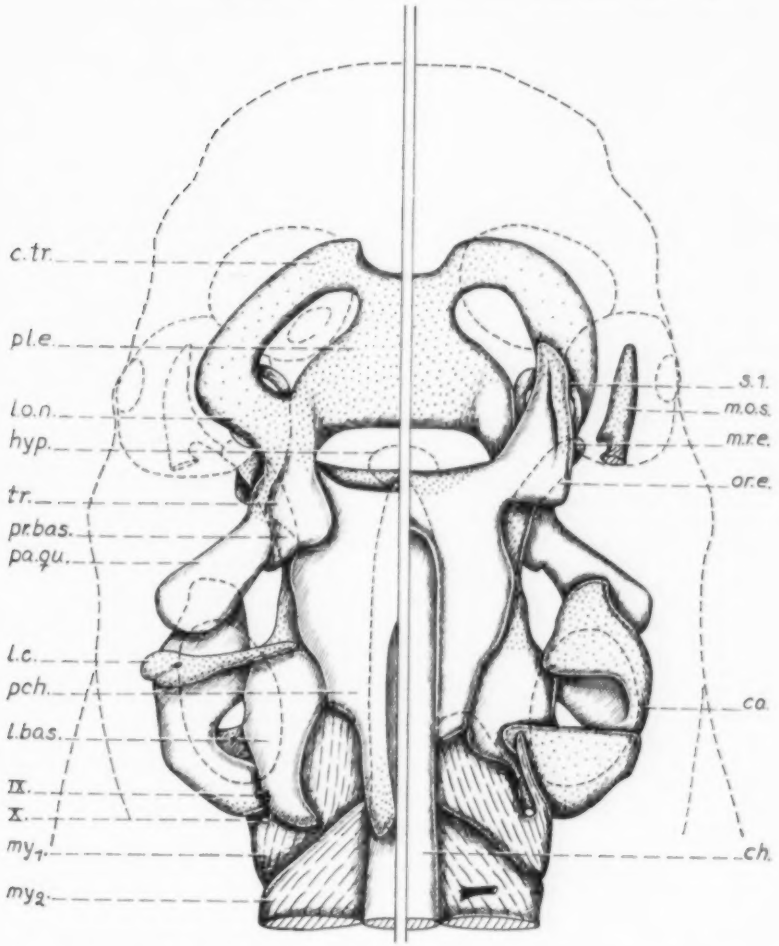

Fig. 51. *Neoceratodus forsteri*, 11.5 mm. Reconstruction of neural chondrocranium and palatoquadrates. Left, ventral view; right, dorsal view. Indications as in Figs. 1—2, 13. 63 ×.

and from behind. The basal portions of these processes are, however, dissolving already at the 10.6 mm stage (Fig. 50). It is not the author's intention to deal with the problem concerning the homologization of these processes. However, several circumstances point towards the possibility that they may represent rudimentary hyal and first metotic neural arches, e.g. their morphology and relations to parachordals—probably fused sclerotomes—brain, and dorsal nerve roots*.

* The orbital cartilages (alisphenoidplatten SEWERTZOFF 1902) may for similar reasons possibly represent the neural arches of the mandibular segments.

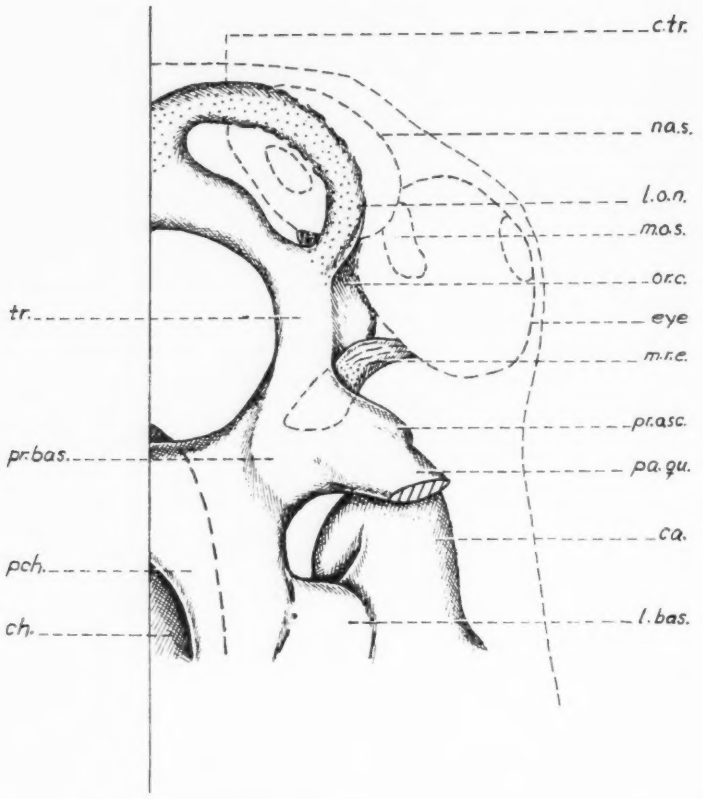

Fig. 52. *Neoceratodus forsteri*, 12.6 mm. Reconstruction of anterior part of chondrocranium, ventral view. Indications as in Figs. 1—2, 33. 63 ×.

If that is the case *Neoceratodus* is, in this respect, a unique and very primitive fish. Its age makes such a possibility quite reasonable.

As usual the floor of the otic capsules is medially connected with the basiotic laminas by means of the anterior and posterior basicapsular commissures (a.bc.c., p.bc.c.) and the basivestibular commissures (bv.c.). The demarcation lines towards the laminas are quite distinct both morphologically and histologically (the otic cartilage floors are ectomesenchymal, whereas the basiotic laminas are endomesenchymal).

At the 11.5 mm stage also the basiotic laminas and otic cartilages are chondrifying (Fig. 51). There is yet a distinct difference between separate elements. Even in late chondral stage the different elements are discerned in the same way as in acipenserids and characids (cf. p. 272). The basiotic laminas are caudally blastemal also at the 11.5 mm stage and connected with the first occipital der-

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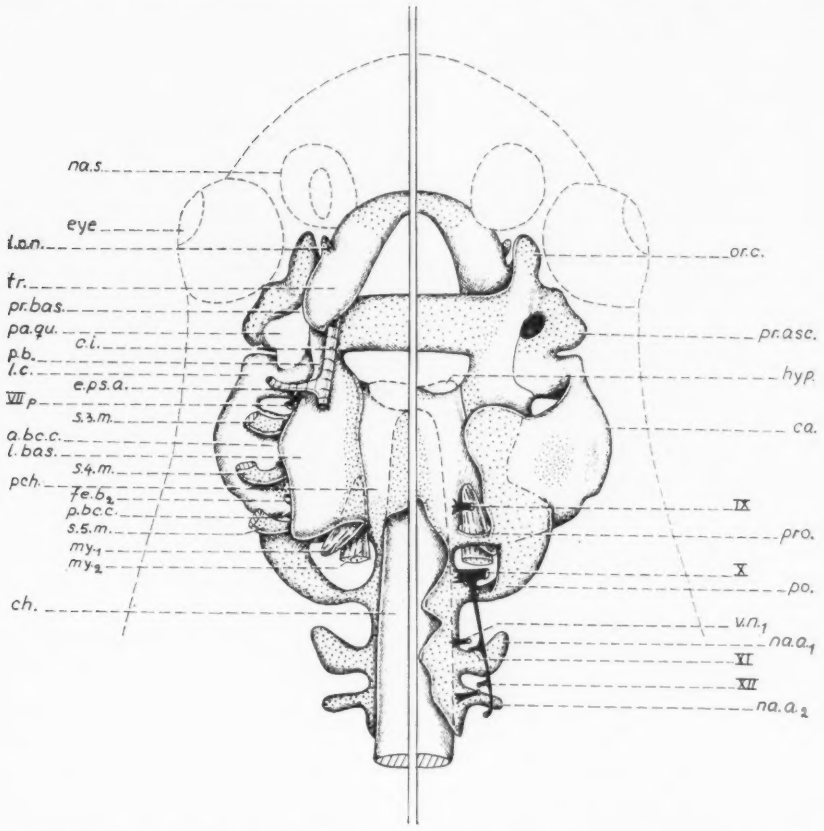

Fig. 53. *Protopterus annectens,* 14 mm. Reconstruction of neural chondrocranium and palatoquadrates. Left ventral view, right dorsal view. Indications as in Figs. 1—2, 13. 38 ×.

mo-myotomes. The dermatomic mesenchyme later forms connective tissue. Rostrally the external rectus muscles (m.r.e.) differentiate close to the eyes, whereas their posterior portion partly dissolves, partly penetrates between the caudal end of the trabecles and the basal process of the quadrates (Fig. 52). Their former connection with the basiotic laminas dissolves.

In short it is obvious that the basiotic laminas in *Neoceratodus* reveal many similarities with those of *Acipenser*. They are formed in three portions ("mesenchyme somites") from the lateral part of the unsegmented otic mesoderm, situated ventromedially to the otic vesicles and in a position corresponding perfectly to the dermo-myotomes of the occipital region. They also initially form a direct posterior connection with these dermo-myotomes. Even the further differentiation is similar, as they chondrify in one piece and simultaneously with the otic car-

tilages but later than the parachordals (sclerotomes). The demarcation lines against the parachordals and the commissures of the otic cartilages are still discerned at the late chondral stage, whereas the initial ventrolateral connections with the mesoderm of the hyal and first and second branchial arches dissolve. The difference between *Neoceratodus* and *Acipenser* in this connection lies in the fact that in the former the basiotic laminas are initially broadly connected with the rectus externus muscle rudiments (Fig. 49). This connection, however, dissolves when muscle tissue differentiates.

Early embryonic stages of *Protopterus* were available but not of *Lepidosiren*. Judging from the material at the author's disposition it seems probable, however, that *Lepidosiren* and *Protopterus* are alike in this respect. *Protopterus* has basiotic laminas formed in principally the same way as in *Neoceratodus* (Fig. 53). As to their rostral extension, however, they differ. As is the case in actinopterygians except the acipencerids the basiotic laminas in *Protopterus* pass the tip of the notochord and the hypophysis. Whether their rostral part is a polar cartilage or not is difficult to decide, as the somites in *Protopterus* are badly delimited.

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In summary the preceding account has shown (1) that *basiotic laminas* actually form *also in dipnoans* and *lower actinopterygians;* they are hence not only found in selachians and higher actinopterygians as was believed earlier (cf. p. 261). It has furthermore revealed (2) that the endomesenchymal basiotic laminas also in dipnoans and lower actinopterygians are primarily formed in connection with the mesoderm (endomesenchyme) of the hyal and first and second branchial arches, (3) that the basiotic laminas at these junctions are thicker and have a constant relation to the dorsal nerve roots, (4) that the basiotic laminas are, even after the chondrification, possible to discern from parachordals and otic cartilages, (5) that the basiotic laminas in dipnoans and lower actinopterygians do not dominate over the parachordals as they do in higher actinopterygians (p. 267) and, finally, (6) that the basiotic laminas may represent transformed dermo-myotomes, as was suggested by the author (p. 268).

This investigation of lower actinopterygians supports the theory proposed above (p. 268) that the serial formation of the teleostomian basiotic laminas corresponds to an old segmentation scheme (metamerism) of the otic region. In elasmobranchs they are formed only from two somites, the hyal and first branchial ones (p. 260), whereas in teleostomians (and dipnoans) also the *second* branchial "mesenchyme somites" take part in their formation. A segmentation of the teleostomian skull is recently suggested by Jarvik (1959) on a paleontological basis.

When finally considering the origin of the basiotic laminas in selachians, it has been mentioned (p. 261) that in *Squalus acanthias* 7—8 mm the basiotic laminas are, according to Holmgren (1940), formed from the lateral part of the sclerotomes but located as myotomes. Then "also the myotome portion transforms into

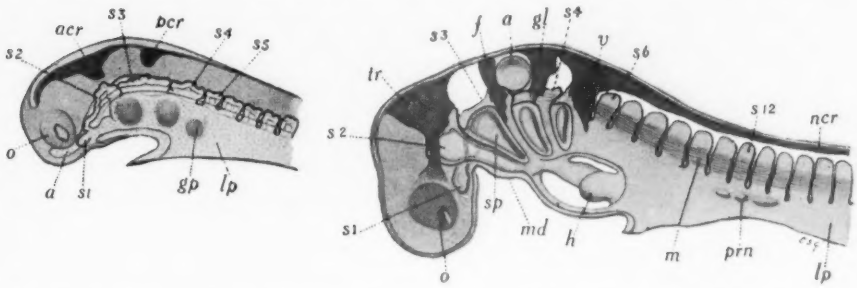

Fig. 54. *Scyllium canicula*, left-side view of anterior region of young embryos. A, stage F; B, 5 mm, stage G. From GOODRICH 1930, Figs. 236, 237). *gp*, hypoblastic gill-pouch; *lp*, lateral plate mesoblast; *sp*, first or spiracular gill-slit; *s 1—5*, first to fifth mesoblastic somites; neural crest is shown darkly shaded. For further letterings cf. GOODRICH.

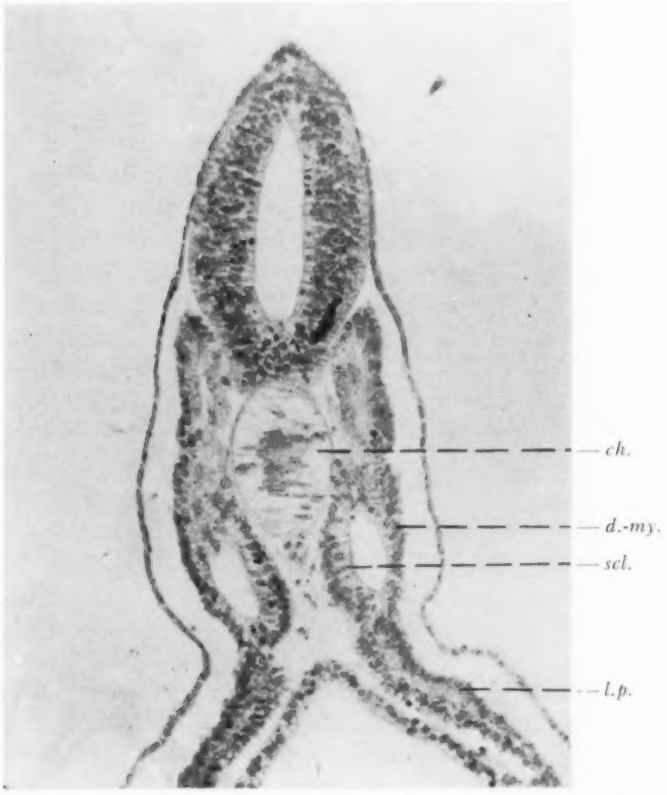

Fig. 55. *Squalus acanthias*, 4.5 mm. Transverse section of trunk to show structure and position of the somites. 160 ×.

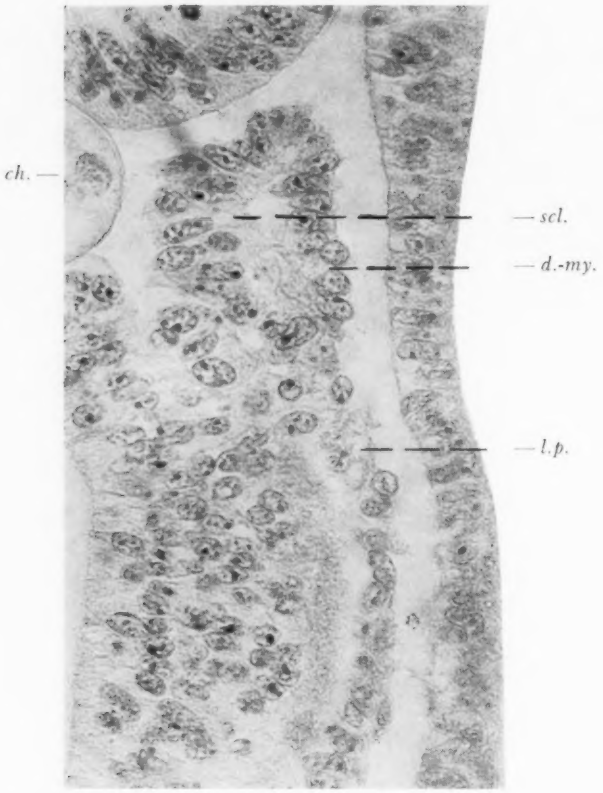

Fig. 56. *Squalus acanthias*, 4.5 mm. Transverse section through right part of skull to show structure and position of the first branchial (metotic) somite. 325 ×.

skeletal tissue" in these somites. Also at the 12.5 mm stage the same thing was suggested. These rather contradictory statements have caused the present author to re-examine HOLMGREN's material (cf. p. 205) and then to extend the investigations to younger stages.

Summarily it has been found that the head is segmented to a point just behind the hypophysis, as was suggested by BALFOUR, VAN WIJHE, GOODRICH, HOLMGREN and others. The mesoderm becomes subdivided into dorsal somites and a ventral unsegmented lateral plate (Figs. 54 A, B). The head somites are of the same nature as those of the trunk (Fig. 55) and form a continuous series with them (cf. VAN WIJHE 1882, et al). Each somite consists of a medial sclerotome (scl.) and a lateral dermo-myotome (Fig. 55, d-my) (cf. VAN WIJHE 1882). The primitive uniform disposition of the somites is disturbed by the invagination of the otic vesicles and for that reason, probably, the first branchial (first metotic) somites never become as large as the others (Fig. 56).

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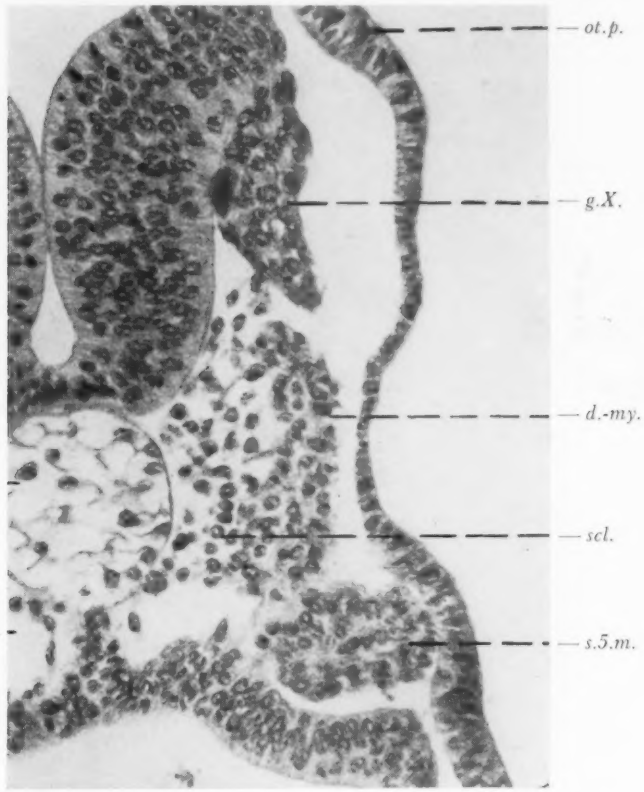

Fig. 57. *Squalus acanthias,* 6 mm. Transverse section through right part of skull, on a level with the second branchial somite (the most anterior of the trunk somites). 272 ×.

At the 6 mm stage the dermo-myotomes of the first and second branchial somites are turning mesenchymal and the head cavities are vanishing (Figs. 57—60). As is seen in Figs. 57—58 mesenchymal cells proliferate from the dermatomes. They will later form the dermis. The remaining part of the first branchial dermo-myotomes take part in the formation of the posterior portion of the *basiotic laminas,* whereas the first branchial sclerotomes contribute to the parachordals only*. The anterior part of the basiotic laminas are formed from the ventral part of the hyal dermo-myotomes (Fig. 59). Anterodorsally there is a hyal head cavity left (s. 3)**. From this portion of each hyal somite the m.rectus externus later

* The reason why HOLMGREN suggested the sclerotomes to form the basiotic laminas seems to be that at the stage, where he mentions them for the first time (7–8 mm), the ventral portion of the dermo-myotomes are already partly mesenchymal and then not so easy to distinguish from the sclerotomes. In order to trace the origin of the laminas it is necessary to go back to younger stages than HOLMGREN did.

** As to the discussion that the "prootic head-cavities" are secondary cf. WEDIN 1948. This problem is not dealt with in this account.

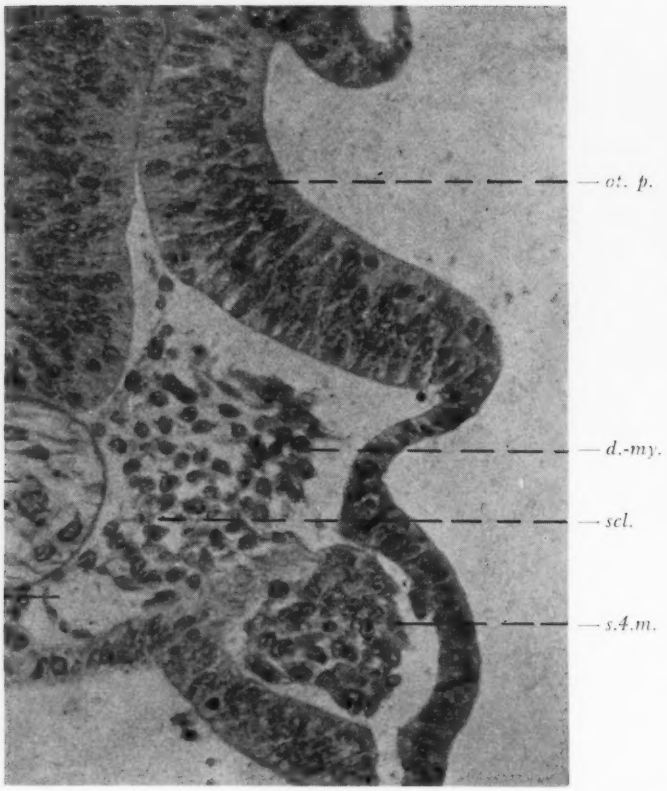

Fig. 58. *Squalus acanthias*, 6 mm. Transverse section through right part of skull, on a level with the first branchial somite. In this and Fig. 57 parts of the dermatomic mesenchyme is about to detach.

will differentiate. In a horizontal section (Fig. 60) it is illustrated how the dermomyotomes (basiotic laminas) of the branchial somites reach the hyal somites between their sclerotomes (parachordals) and the otic vesicles.

The further differentiation of the basiotic laminas is described in HOLMGREN's paper (1940) and shown in Figs. 61, 62. Generally speaking it coincides with that of teleostomes and dipnoans, and therefore it shall not be dealt with here.

The question what the basiotic laminas represent and how they are formed phyletically seem to have come to a possible solution through this survey. Their genesis in selachians, dipnoans and acipenserids lends support to the theory that they represent most of* the posterior part of the hyal plus most of the first (in selachians) or the first and second (in teleostomes and dipnoans) metotic (or

* Parts of the dermatomes continue their normal task to contribute mesenchyme for the dermis, cf. p. 283 and Figs. 56—60.

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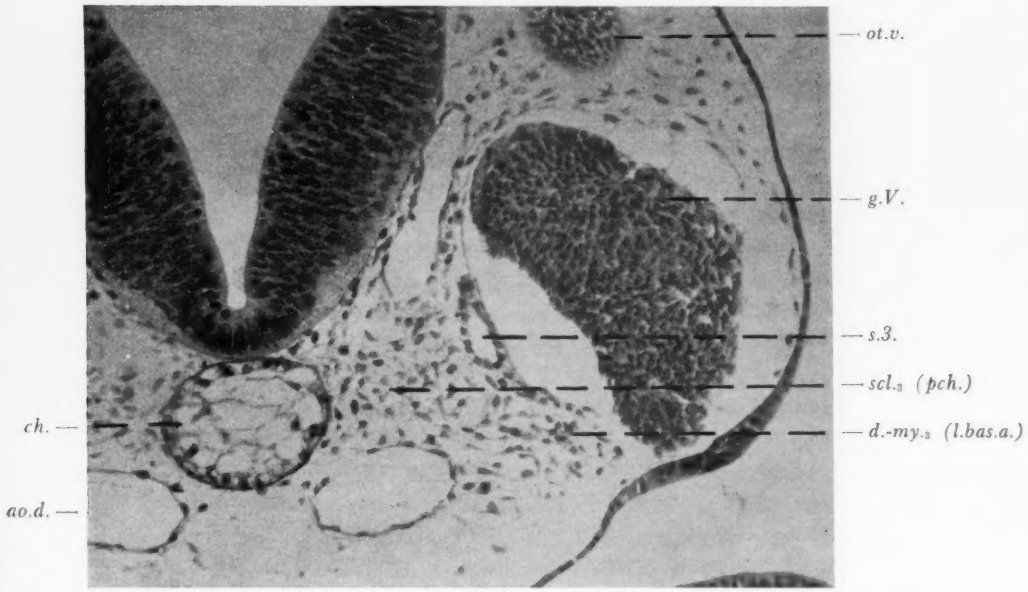

Fig. 59. *Squalus acanthias*, 6 mm. Transverse section through right part of skull, on a level with the hyal somite. 200 ×.

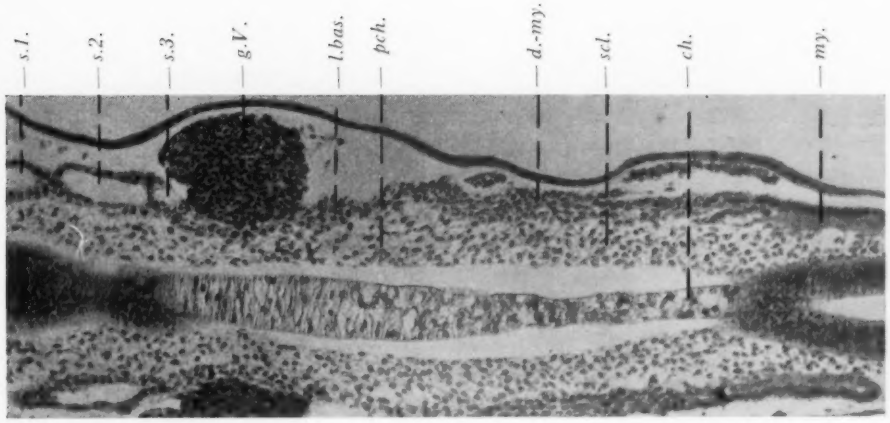

Fig. 60. *Squalus acanthias*, 6 mm. Horizontal section of skull and anterior part of trunk. The sections shows that the trunk somites proceed into the skull and reach the prootic somites. 110 ×.

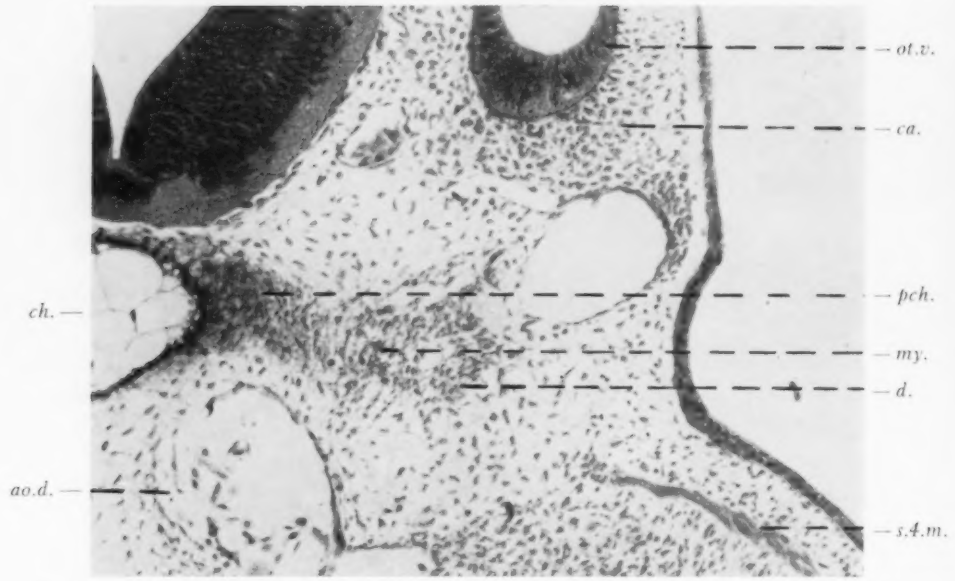

Fig. 61. *Squalus acanthias*, 17 mm. Transverse section through right part of skull to show the fate of the sclerotome and dermo-myotome of the second branchial somite (cf. Fig. 57). 150 ×.

branchial) d e r m o-m y o t o m e s. Anteriorly and posteriorly to the otic region the myotomes form muscle tissue as usual.

A plausible explanation of the transformation of the dermo-myotomes only in the vicinity of the otic vesicles might be that the somites of this region originally were engaged in the protection of the inner ear and the brain. In order to better fulfil this function, the dermo-myotomes also got potency to differentiate into skeletal tissue, which gradually developed into the present chondral basiotic laminas.

According to this theory the fate of the different parts of the somites are not fixed once and for all. This conception, however, is not new. In sharks and amphibians (Nelsen 1953) and usually also in chick (Williams 1910, Nelsen 1953) *sclerotomic* mesenchyme appears to contribute to *dermal* areas. And in regenerating urodele tail *muscle* tissue dissolves into blastema which then forms *cartilage* and connective tissue (Holtzer 1956).

As a parallel it might be mentioned that the ectomesenchymal cell-laminae deliminated in the sharks (Holmgren 1940) and other gnathostomians (Jarvik 1959; Ørvig 1951) obviously form various skeletal tissues (dentine, bone, cartilage, connective tissue), "and the various types of these categories are thus fundamentally of the same nature and they may pass over gradually into each other or may be linked by tissues intermediate in structure" (Jarvik 1959, p. 47).

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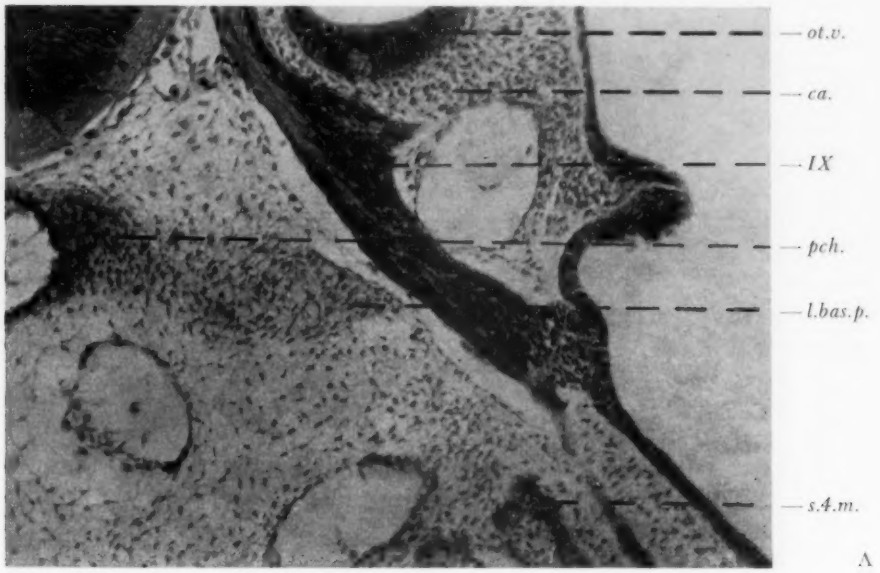

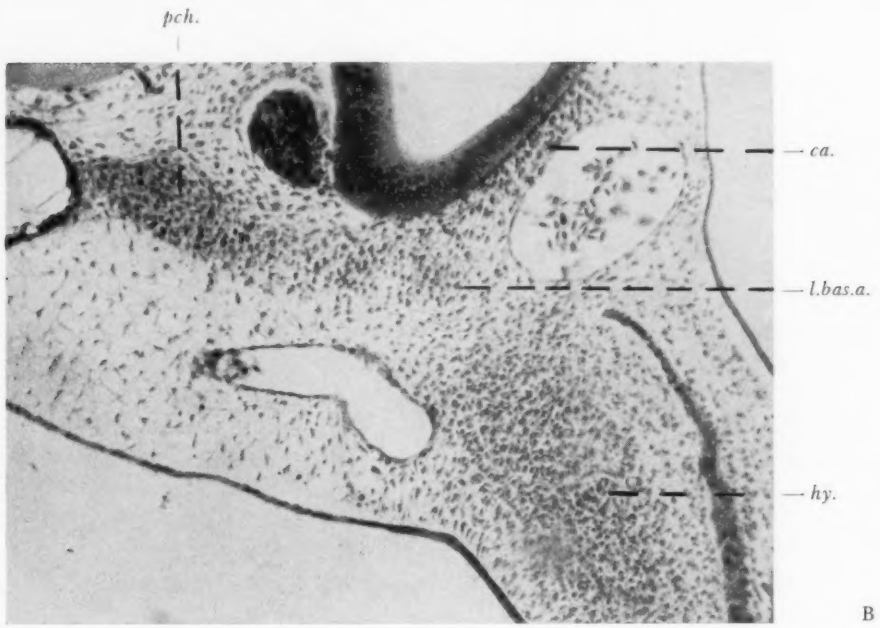

Fig. 62. *Squalus acanthias*, 20 mm. Transverse section through right part of skull to show the fate of A, the first branchial somite (cf. Fig. 58) and B, the posterior part of the hyal somite (cf. Fig. 59). 150 ×.

POLAR CARTILAGES

The polar cartilages were discovered by NOORDENBOS (1904) in the rabbit. In the same year (and later also in 1922) VAN WIJHE found also in *Squalus acanthias* independent cartilage elements situated between the "parachordals" and the trabecles, i.e. where NOORDENBOS found the polar cartilages in the rabbit. Later on SONIES (1907) found independent polar cartilages in birds, VEIT (1911) and HAMMARBERG (1937) in *Lepisosteus* and HOLMGREN (1943) in *Amia**.

Neither in Petromyzon nor in any other agnath separate polar cartilages are described, but JOHNELS (1948) observed that the posterior parts of the trabecles "develop in the mandibular segment and should, thus, be homologous with the polar cartilages of Gnathostomes".

Separate polar cartilages have not been described in the teleosts either. As late as in 1958 DEVILLERS suggested that in the majority of the teleosts "le trabécule se soude directement au parachordal". In 1943 HUBENDICK had noted, however, that the enlarged posterior portion of each trabecle in *Leuciscus* has exactly the same position as the polar cartilages in *Lepisosteus,* i.e. it is situated between the connection of the carotis interna and the efferent pseudobranchial artery, ventrally, and the m.rectus externus, dorsally. He therefore suggested that this portion corresponds to a polar cartilage.

A comparison with *Hepsetus* and *Salmo* (cf. chapter III and below) strengthens this suggestion: HOLMGREN (1943) found polar cartilages at the blastema stage of *Salmo salar,* only loosely connected with the caudal end of each trabecle but also "undoubtedly connected with the mandibular somite, from which it has probably arisen. It is not possible, however, to determine this derivation with absolute certainty, as the mandibular somite is but diffusely outlined".

At the blastema stage (6.5—7.3 mm) polar cartilages are formed also in *Hepsetus.* They are densely nuclified and by means of a thin and sparsely nuclified blastemal zone connected with the trabecles and the basiotic laminas (Figs. 1, 2, 7,8). As in *Salmo* and *Holostei* the polar cartilages in *Hepsetus* are also situated between the m.rectus externus, dorsally, and the point where the efferent pseudobranchial artery joins the carotis interna, ventrally (cf. p. 211). When the polar cartilage rudiments later chondrify, the demarcation lines towards the trabecles and the basiotic laminas disappear. Morphologically, however, the polar cartilages can be traced, in the horizontal plane, as a caudal hook of the S-shaped trabeculopolar bar (Fig. 16) and, in the lateral plane, as the horizontal caudal part of the same bar (Fig. 15). At the 12.6 mm stage the chondrocytes at the border against the trabecles are dissolving (Figs. 25, 27), with the result that at the 23 mm stage the polar cartilages are free from the trabecles and only connected with the basiotic laminas (Figs. 33, 34). The mm. recti externi never insert on the polar car-

* PEHRSON (1922) had earlier suggested them to represent the connection between the trabecles and the "parachordals". HOLMGREN, however, studied younger embryos.

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tilages but lie in the close vicinity of them; at later stages they grow caudad and insert in the posterior myodome (p.my.).

When trying to settle the origin of the polar cartilages neither the teleosteans nor the holosteans are suitable, as the prootic mesoderm is not distinctly segmented. Such qualifications have, however, *Acipenser* (among the teleostomes) as well as selachians and (partly) dipnoans.

The *Selachii* are best investigated in this respect (ALLIS 1923, 1931; HALLER 1924; MATVEIEFF 1925; DE BEER 1931 b; and HOLMGREN 1940, 1943). Among these authors ALLIS suggested the polar cartilages to be of visceral origin; their relation to the carotis interna was taken as a criterion that they are a pharyngomandibular element. From the material at his disposal DE BEER was not able to obtain a satisfactory verification of ALLIS' hypothesis but suggested that the polar cartilages at least arise from the mandibular arches. According to HALLER each polar cartilage is formed in the "Hypophysenpolster", the origin of which was, however, not determined. Yet it was said to be connected with the posterior portion of the mandibular rudiment.—MATVEIEFF was the first to show ontogenetically that the polar cartilages are not of visceral but of somitic origin. He suggested that the hyal somites supply material partly for the polar cartilages and partly for the anterior part of the parachordal plate. In opposition to that, HOLMGREN (1940) in a very detailed study of early stages of *Squalus acanthias* and other selachians found that the polar cartilages together with the acrochordal tissue instead detach from the medial wall of the *mandibular* somites.

Acipenser has, in this respect, been studied by three authors: MATVEIEFF (1925), SEWERTZOFF (1928) and HOLMGREN (1943). All three suggested they had found polar cartilages, but these were considered not to be separately formed. Actually the details of the early development of the polar cartilages are not very well known and the data are partly contradictory. MATVEIEFF concluded that the polar cartilages are formed from the anterior sclerotome of the hyal somites (cf. his opinion of the selachians, above) and are represented by the thick anterior end of the parachordals. As, however, his investigation begins with an 11 mm stage—at which chondrification has already begun—his conclusion seems to be based on very uncertain evidence. SEWERTZOFF, on the other hand, considered the polar cartilages to represent the posterior, separately chondrified part, of the trabecles, but he did not take into consideration their somitic origin. Holmgren examined a large material of young stages of *Acipenser ruthenus, A.güldenstaedti* and *A.stellatus*. He then found that the polar cartilages and the acrochordal tissue are detached from the medial wall of the mandibular somites just as in selachians. The acrochordal tissue emanates first and later chondrifies at the tip of the notochord. According to HOLMGREN it hence represents SEWERTZOFF's anterior parachordals and MATVEIEFF's polar cartilages. Contrary to the conditions in selachians it is permanently connected with the polar cartilages. These grow forwards, according to HOLMGREN probably to the carotid incissure, i.e. about to the point

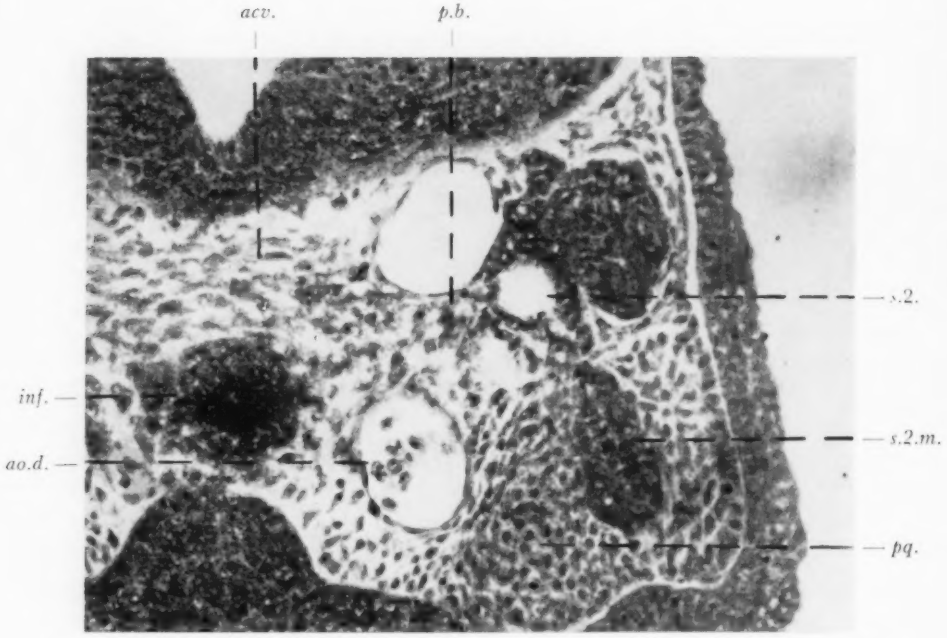

Fig. 63. *Acipenser güldenstaedti*, 7 mm. Transverse section through right part of skull showing the polar cartilage cells detaching from the medial wall of the mandibular somite. Mesoderm and endomesenchyme are laden with yolk. 246 ×.

where MATVEIEFF draws the demarcation line between "the sclerotomes II,2s and II,1s" and to the point where SEWERTZOFF suggests the border line between the trabecles and the polar cartilages to be situated.

However, the descriptions of HOLMGREN and the other authors are rather short and not accompanied by any illustrations. The present author has re-investigated HOLMGREN's material in order to get better information on this point.

As HOLMGREN shortly mentioned from *A.ruthenus* the rudimentary brain flexure in young stages of *Acipenser* contains cells detaching from the medial surface of the posterior part of the mandibular somites. This comparably important observation can be seen in Fig. 63 of *A.güldenstaedti*. The cells detaching from the medial portion of the mandibular somites (s.2) spread mediad between the hypophysis and the tip of the notochord. Later on these cells are grouped into two densely nucleified portions (cf. *A.ruthenus* 8.6 mm, Fig. 45 A), one medial part below the tip of the notochord, the acrochordal tissue (acr.), and one lateral part connected with the mandibular somites which are still intact (s.2). These cells are the rudiments of the polar cartilages (p.b.). Caudally the polar cartilages connect with the basiotic laminas (l.bas.) by means of a loose mesenchyme, whereas the acrochordal caudally is still free from the parachordals (pch.). From this stage

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it should finally be mentioned that each polar cartilage is laterally connected with the m.rectus externus (m.r.e.) and situated dorsally to the point where the carotis interna (c.i.) joins the efferent pseudobranchial artery (Fig. 45 A).

In *A.stellatus* 10 mm (Fig. 45 B) the premandibular commissure (Fig. 45 A, s.l.com.), which was still intact in the former stage, is dissolving, its cells intermingling with those of the acrochordal tissue (Fig. 45 B, acr.). The mandibular somites have almost lost their cavities (s.2.) and their walls are beginning to produce myoblasts for the m.obliquus superior (m.o.s.). At early stages even the hyal somites have head cavities (Fig. 67), hithertoo not described in *Acipenser*. In *A.stellatus* 10 mm these cavities have been replaced by myoblasts for the m.rectus externus (Fig. 45 B, m.r.e.). The polar cartilages (p.b.) are still blastemal, whereas the acrochordal tissue is prochondral. This element has caudally joined the parachordals (pch.) growing rostrad. The connection between the polar cartilages and the basiotic laminas remains but is narrowing and disintegrating. As in teleosts the r.palatinus facialis (VII_p) runs at the posterolateral border of each polar cartilage. Anteromedially the polar cartilages have now joined the medial part of the trabecles (cf. *Hepsetus* stage I) (tr.i.). Still both are discernible, however, as the rounded cells of the polar cartilages are concentrically grouped into a cord, whereas those of the medial part of the trabecles are flattened and form a plate. The well-known carotid incissure (cf. above) represents the border between them. This stage seems to correspond to the youngest stage described by SEWERTZOFF ("a very young larva"), though no separate chondrification of the trabecles and the polar cartilages is to be seen (cf. p. 289).

In *A.stellatus* 13 mm (Fig. 46) the polar cartilages are still blastemal although completely separated from the basiotic laminas. Their differentiation, however, goes faster in *A.ruthenus*, where already in a 12.5 mm specimen they are beginning to chondrify. To the earlier connection with the medial part of the trabecles is later added a connection also with the lateral part of the trabecles (cf. *Hepsetus* stage II). The carotid foramen then formed (Fig. 46) hence represents the border between the trabecles and the polar cartilages, as was suggested by SEWERTZOFF and HOLMGREN.

In short the present investigation of the origin of the polar cartilages in acipenserids has confirmed HOLMGREN's suggestion that they are, together with the acrochordal tissue, d e t a c h e d from the m e d i a l wall of the m a n d i b u l a r somites. Consequently the polar cartilages and the acrochordal tissue seem to represent the lateral and medial portions, respectively, of the mandibular *sclerotomes*. The only essential difference between acipenserids and selachians (HOLMGREN 1940) in this respect hence seems to be that in selachians the acrochordal tissue forms much earlier than the polar cartilages.

When finally considering the *Dipnoi* no separate polar cartilages are described (SEWERTZOFF 1902; KRAWETZ 1911; GREIL 1913; and others). The present author

has not found any either. Nor has it been possible to decide with any certainty from which prootic somites the rostral skeletal endomesenchyme is derived: the somit borders are usually too diffuse and no typical cell proliferations are found. Judging from the morphology and position, however, the endomesenchyme that in *Neoceratodus* (Fig. 49, acr.) is grouped around the notochord tip above the premandibular commissure (s.l.com.), and that in *Protopterus* is situated anteriorly to the notochord (Fig. 53, acr.), seems to correspond to the acrochordal tissue in selachians and acipenserids. The posteroventral part of that endomesenchyme should then represent the polar cartilage (Figs. 49, 50, 53, p.b.), as it lies above the junction of the carotis interna (c.i.) and the mandibular aorta (ao.md.), and is laterally connected with the m.rectus externus (m.r.e.) (cf. Acipenser, p. 291). If that interpretation is correct the polar cartilages have different types of caudal connections: in *Neoceratodus* they are connected with the parachordals (Figs. 49, 50, pch.) and in *Protopterus* and *Lepidosiren* with the basiotic laminas (Fig. 53, l.bas.).

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Summing up the following may be mentioned concerning the *morphology* of the polar cartilages in fishes. Separate polar cartilages are described in selachians and *Lepisosteus*. They are said to exist as distinct elements also in *Acipenser, Amia, Salmo* and possibly *Leuciscus*. As is now stated, however, *Hepsetus* also has polar cartilages situated in the same position, viz. between the m.rectus externus, dorsally, and the junction between the carotis interna and the efferent pseudobranchial artery, ventrally. An investigation of *Acipenser* has proved the existence of polar cartilages in loose connection with the basiotic laminas, caudally. Contrary to the situation in holosteans and teleosteans they are, however, continuous with an acrochordal tissue, medially, and primarily independent of the trabecles, rostrally. The trabecles later fuse with the polar cartilages, first medially, and then laterally to the carotis interna. A carotid foramen is hence formed on the border between the trabecles and the polar cartilages. In *Dipnoi* on the contrary the present investigation has not shown any separate polar cartilages. Because of comparative anatomical reasons, however, it seems possible that polar cartilages are present, in *Neoceratodus* connected with the parachordals, and in *Protopterus* and *Lepidosiren* connected with the basiotic laminas.

Opinions differ about the *origin* of the polar cartilages. The present investigation of *Acipenser* has shown that HOLMGREN's theory (1943) of a mandibular somite origin is correct, as it has been found that the endomesenchyme of the polar cartilages and the acrochordal tissue detach from the medial wall of the mandibular somites and spread mediad. The polar cartilages and the acrochordal tissue accordingly seem to represent the m a n d i b u l a r s c l e r o t o m e s. In holosts and teleosts no distinct mandibular somites are hithertoo described. The morphological relations of the polar cartilages indicate, however, that they probably have the same origin as in selachians and acipenserids.

TRABECLES

"A question of primary importance to the very theory of the morphology of the skull is that of the origin of the polar cartilages and the trabeculae cranii. Upon the answer of this question depends the conception of neurocranial as well as viscerocranial structures." HOLMGREN's statement 1943 has recently been actualized when JARVIK (1952) in an excellent study of *Eusthenopteron foordi* has suggested the ectomesenchymal part of the trabecles (the suborbital ledge) to be homologous with a visceral element (the infrapharyngomandibula).

The theory of a visceral origin of the trabecles is, however, not new. It has in fact been maintained long ago by morphologists, such as HUXLEY, SEWERTZOFF, ALLIS and DE BEER. After RATHKE in 1839 for the first time had described the trabecles in the grass-snake, HUXLEY (1874—76) studied different animals and then suggested the trabecles to correspond to parts of the visceral skeleton in gnathostomes and to the anterior parts of the skeleton in the cranial base of *Petromyzon* (1876). HUXLEY's opinion was shared by CALBERLA (1877), PARKER (1879, 1883) and others. On the contrary the statement of KOLTZOFF (1902) that the trabecles of *Petromyzon* are primarily of somitic origin led SEWERTZOFF (1916) to deny HUXLEY's hypothesis.

KOLTZOFF's suggestion of a somitic origin of the trabecles in *Petromyzon* was later disproved by DAMAS (1944), who considered them to be derived from ectomesenchyme instead. According to JOHNELS (1948), however, DAMAS seems to have confused the trabecles with the parabuccal cell band. Instead JOHNELS in principle accepted KOLTZOFF's statement and considered the trabecles (including the polar cartilages) to be derived from the first and second segments. However, he admits that the similarities between mesenchyme from the ecto- and endomesoderm makes it very difficult to draw safe conclusions from the material.

This admission is to be (or should be) repeated when discussing the origin of the gnathostomian trabecles. Of those fishes that have distinct head somites *Squalus acanthias* has been the subject of particular interest. According to HALLER (1924) the anterior part of the "trabecles" (trabeculae sensu stricto) is derived from the maxillary process of the mandibular arch. DE BEER (1931 b) had about the same opinion, as he found that the trabecles and mandibular arches are primarily joined at their anterior ends. For this and other reasons he suggested the trabecles to belong to the premandibular arches. A similar theory had, however, earlier been given by ALLIS (1923, 1924), who, on a comparative anatomic basis, suggested them to be a pharyngopremandibular element.

MATVEIEFF (1925), on the other hand, had the opposite opinion: the mandibular and premandibular somites furnish material for the posterior part of the trabecles, whereas the anterior part is formed from mesenchyme of another origin. HOLMGREN (1940, 1943), who had access to younger embryonic stages both of *Squalus* and several other selachians, came, however, to another conclusion. He found that the posterior parts of the trabecles certainly are somitic but derived

from only one somite, the premandibular. The mandibular somites instead give rise to the polar cartilages (p. 289). Furthermore HOLMGREN found that the rostral part of the trabecles are formed from ectomesenchyme.

In fact, however, HOLMGREN was far from certain of the somitic origin of the trabecular rudiments: "These are questions that need further investigations" (1940, p. 67). He also admitted that it was impossible to say with certainty if the entomesodermal "cell band" (the trabecular rudiment), which at the 12.5—13 mm stages caudally borders the vesicle of PLATT really is the first separate trabecular rudiment, because "already in these young stages there are indications that also ectomesodermic material could enter the trabecular rudiment" (1940, p. 67).

HOLMGREN later (1943) extended his studies also to early stages of *Acipenser*, *Amia* and *Salmo*. Different species of *Acipenser* had earlier been investigated because of their distinct prootic somites (DE BEER 1925, MATVEIEFF 1925, SEWERTZOFF 1928 and NEUMAYER 1932). *Amia* had been examined by PEHRSON (1922) and *Salmo* by e.g. DE BEER (1931 b). For further information on these investigations is refered to HOLMGREN's work. HOLMGREN's investigation of a large material gave results that did not tally with those of the other authors. He found that the trabecle rudiments in *Acipenser* are caudally indented by the carotis interna (cf. *Hepsetus* stage I—III and p. 289), and that they probably are of axial origin but formed only from the premandibular somites.—*Amia* and *Salmo* have very diffuse prootic somites and are therefore more difficult to study in this connection. The material of *Amia* "did not allow any close analysis of the origin of the trabecles", but probably there is no principal difference between *Amia* and *Acipenser*, HOLMGREN adds. In any case, the mesenchymal lamella in *Amia*, extending rostrad from the polar cartilages medially to the carotis interna, should according to HOLMGREN "answer to the first trabecular rudiment in *Acipenser*" (i.e. the medial part of the adult trabecle). This lamella later chondrifies as a rostral process of the polar cartilages (cf. PEHRSON 1922) and serves as a fastening for some of the eye muscles. — As to *Salmo salar* HOLMGREN concludes that "the trabecles are developing from the premandibular somite" (1943, p. 15). Earlier (l.c., p. 14) he admits, however, that "there thus exists a possibility that ectomesodermal cells could take part in the formation of the most anterior portion of the trabecular system." Actually, if the diffuse shape of the prootic somites and the great similarities between endo- and ectomesenchyme are kept in mind, there seems to be every reason not to rely too much on anatomical investigations of so comparatively advanced fishes as holosts and teleosts. Experiments might possibly be more useful for this purpose.

No other thorough studies of the origin of the teleostean trabecles have been published. It could, however, be mentioned as a memento that BHARGAVA recently found (1958) that the trabecles in *Mastacembulus armatus* "become distinct from *the rest of the ectomesenchymatous cells** below the eyes just behind the

* Italics by the present author.

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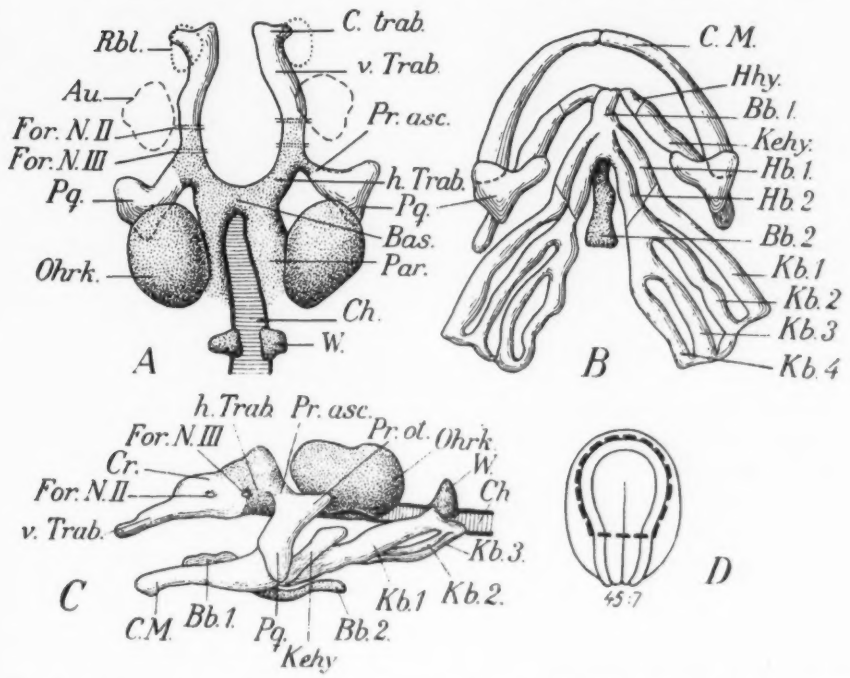

Fig. 64. *Ambystoma mexicanum*. Reconstruction of chondrocranium of a larva. A, neurocranium and palatoquadrates, dorsal view; C, chondrocranium from the left. Cartilage of endomesodermal origin dotted. From HÖRSTADIUS and SELLMAN (1946, Fig. 1). For letterings cf. HÖRSTADIUS and SELLMAN.

hypophysis," indicating a completely ectomesenchymal origin of the trabecles. No details are, however, given.

The ontogeny of the chondrocranium in *Polypterus* is not completely known as mentioned (p. 273). The earliest stage investigated, 6.75 mm, shows free trabecles situated far apart and anterior to the tip of the notochord.

In *Dipnoi* GREIL (1913) has in *Neoceratodus* described separately formed trabecles which later fuse with the parachordals; they are said to be constituted by paraxial mesoderm (l.c., pp. 1068, 1128). When he, however, also considers e.g. the processus basalis of the palatoquadrate to have the same origin (l.c., p. 1068) his observations on this point should be taken with reservation. In *Protopterus* and *Lepidosiren* separate trabecles have not been described (WINSLOW 1898; AGAR 1906; and others).

HOLMGREN's statement (1940, 1943) that the trabecle rudiments grow forwards by means of ectomesenchyme had long ago been suggested by *experimental embryologists*. When KASTSCHENKO (1888) and later GORONOWITSCH (1892, 1893) claimed that some of the mesenchyme of the head originates from ectoderm (the neural

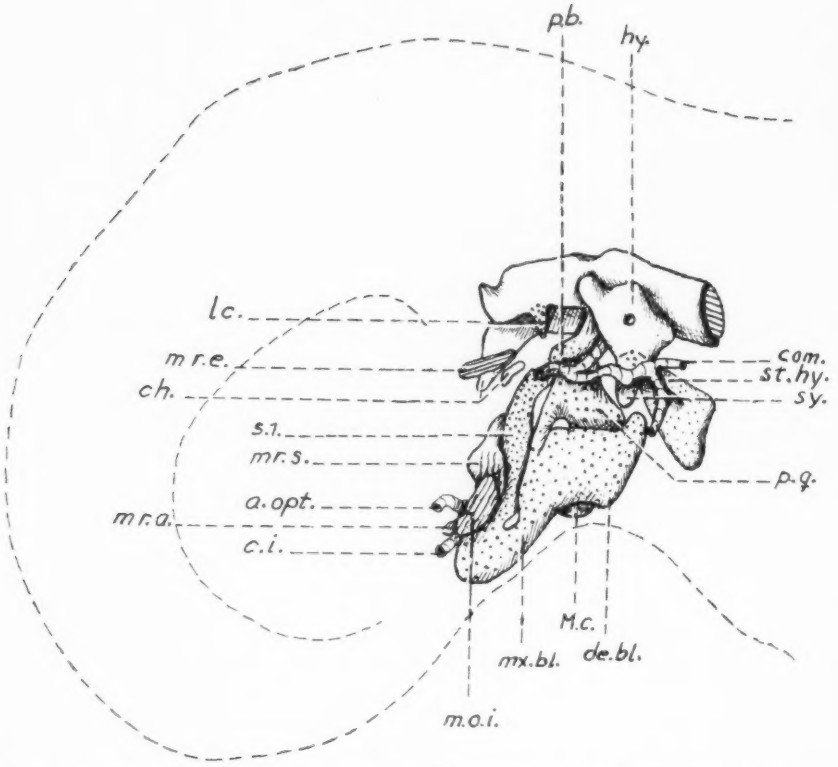

Fig. 65. *Esox lucius*, 8.5 mm. Reconstruction of chondrocranium, lateral view. Indications as in Figs. 1—2, 13. 120 ×.

crest) in selachians, teleosts and birds, an intense discussion started. In 1893—1897 PLATT made an important discovery when she found that the ectoderm produces the mesenchyme building up the cartilage of the visceral arches. In the nineteen-twenties the neural crest problem was clearly analyzed, particulary by LANDACRE (1921) in a morphological study of *Ambystoma jeffersonianum* and by STONE (1922—1929) in an experimental work on the frog. Other authors on this subject are for instance DOHRN, VEIT, NEWTH, KNOUFF, RAVEN, HOLMDAHL, HÖRSTADIUS & SELLMAN. An excellent summary is given by HÖRSTADIUS (1950). All these authors came to the conclusion that "the conditions seem to be similar in all groups of vertebrates. Mesenchyme emerges from the neural crest and migrates ventrally (l.c., Fig. 17), forming the visceral arches and also the anterior part of the trabeculae cranii" (HÖRSTADIUS op. cit.).

However, as to the amphibian neural cranium, neither the morphologists (DOHRN, LANDACRE, VEIT) nor any of the experimental embryologists seem to have contributed to the knowledge of the origin in details of the e n d o m e s o-

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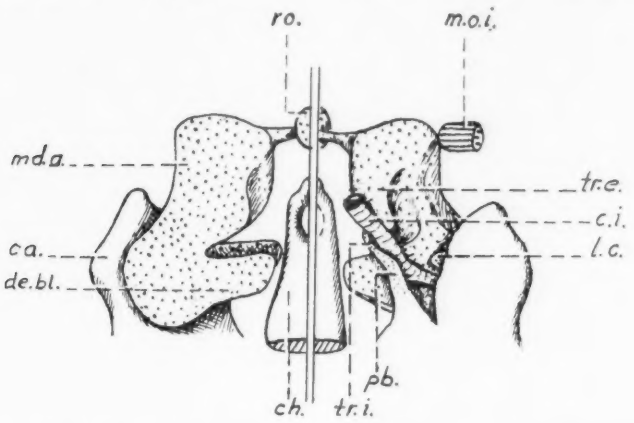

Fig. 66. *Esox lucius,* 8.5 mm. Reconstruction of anterior part of chondrocranium. Left, ventral view; right, dorsal view. Indications as in Figs. 1—2. 125 ×.

d e r m a l elements. No definition was given of what "the posterior part of the trabecles" really meant, from which somite(s) they emerge and so on. A look at the "map" of *Ambystoma* presented by HÖRSTADIUS & SELLMAN (Fig. 64) does not exclude the possibility that they actually correspond to the polar cartilages. Such a notion is strengthened by DE BEER's (1947) morphological study of *Ambystoma,* in which animal (l.c., p. 384) "the line of junction between the yolk-free pigment-containing anterior part of the trabecula and the yolk-containing pigment-free posterior part of the trabecula and the parachordal is seen to be slightly anterior to the posterior wall of the infundibulum." The posterior part of the trabecula is then situated in the region where the *polar cartilage* is formed in fishes. In this connection the morphological investigation of STÖHR should also be pointed out (1879), where the trabecles in *Ambystoma* are said to be formed of mesenchyme that lacks yolk globules, i.e. according to DE BEER's terminology of *ecto*mesenchyme (DE BEER 1947; LANDACRE 1921). On both sides of the tip of the notochord a pair of "Balkenplatten" (anterior parachordals GAUPP 1905; probably = *polar cartilages* according to GOODRICH 1930) simultaneously differentiate from mesenchyme that is said to be of another histological structure, i.e. probably *endo*mesenchyme. The histologically and morphologically different elements, the trabecles and the polar cartilages (if GOODRICH was right), soon grow towards each other and fuse. Principally the same ontogeny is present also in anurans (STÖHR 1881; GAUPP 1893, 1906; SPEMANN 1898; DE BEER 1926). There is thus ample histological and morphological evidence to support the assumption that the "posterior part of the trabecles" in amphibians in reality correspond to the polar cartilages in fish and that the a m p h i b i a n t r a b e c l e s accordingly are not at all dual in origin but c o m p l e t e l y e c t o m e s e n c h y m a l. To

settle this problem definitely, however, a careful study of the origin of the endomesodermal parts of the amphibian neural cranium is needed.

In *Hepsetus* the posterior parts of the trabecles are certainly formed in connection with the posteroventral margin of the premandibular somite vestigies (p. 209, Figs. 3, 7), but, as no distinct histological difference between endo- and ectomesenchyme has been found (cf. p. 208), no definite conclusions can be drawn from this circumstance. The connection can be secondary, as in *Acipenser* and *Neoceratodus* (cf. below). Rostrally the trabecles in *Hepsetus* are primarily continuous with the mandibular arches, so at least this portion seems to be ectomesenchymal. The solution of this problem in teleosts requires experimental studies added to the histological ones. The short paper of LOPASHOV (1944) shows that such studies might be fruitful.

The vestigial premandibular somites in *Hepsetus* can be followed up to the 7.3 mm stage; they consist of a loose tissue inside, which may represent a vestigial head cavity. The other African characid embryos investigated are too differentiated and the American ones too specialized to admit any diagnosis concerning the presence of prootic somites. Of the other teleosts investigated (pp. 205—206) only *Esox* (Fig. 65) possesses such vestigial premandibular somites. But for *Salmo* (HOLMGREN 1943; cf., however, p. 294) no premandibular somites have been described in teleosts. Thus, *Esox, Hepsetus* and *Salmo* so far seem to be primitive in this respect.

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In contrast to *Salmo,* however, *Esox, Hepsetus* and *Corydoras* (HOLMGREN 1943) have trabecles initially connected with the polar cartilages only by means of a blastema lamella (Figs. 2, 3, 66, tr.i.), situated medially to the carotis interna (c.i.). According to the position this lamella seems to correspond to the medial part of the trabecle in *Acipenser* and *Amia* (cf. p. 294) as described by HOLMGREN (1943). If so, the carotid incissure (c.fi.) formed on the border between the trabecles and the polar cartilages should be homologous with the carotid incissure in *Acipenser* (Fig. 45). When the trabecles later join the polar cartilages even laterally to the carotid (stage II), the carotid foramen (Fig. 8, fo.c.) constituted in this manner should accordingly represent the one formed in *Acipenser.* Contrary to *Acipenser,* however, only the lateral connection chondrifies in *Hepsetus Esox* and *Corydoras,* whereas in Amia also a part of the medial connection remains (p. 294). Consequently, there are in teleostomes four types of connection between the trabecles and the polar cartilages:

(1) A blastemal connection on both the medial and the lateral side of the carotis interna; both chondrify*. Probably primitive type. (*Acipenser.*)

(2) D:o; the lateral connection and partly the medial chondrify (*Amia.*)

(3) D:o; only the lateral connection chondrifies. (*Hepsetus, Esox, Corydoras.*)

(4) Only the lateral blastemal connection forms and chondrifies. (Other *Teleostei.*)

* As the medial connection chondrifies earlier, HOLMGREN (1943) considered it to be the trabecula proper.

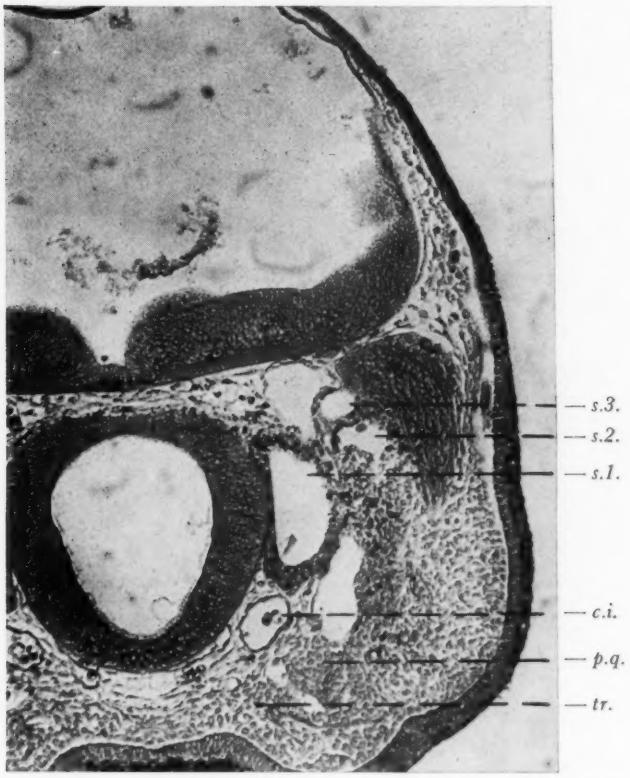

Fig. 67. *Acipenser ruthenus,* 8.6 mm. Transverse section through right part of skull. The section shows the position of the trabecle rudiment and the head cavity present in hyal somite. Mesoderm and endomesenchyme laden with yolk. 220 ×.

When the *origin* of the trabecles in teleostomes is studied from an anatomical point of view *Acipenser* is most suitable to investigate, because this genus has rather distinct prootic somites (Fig. 67), and the endomesenchyme is more yolk--laden (and therefore easier to discern) than the ectomesenchyme*. In spite of this, however, rather different results have been obtained by different authors (cf. p. 289). A reinvestigation of HOLMGREN's material is therefore justified. In short the following results are obtained (Figs. 45—46).

The endomesenchyme in *Acipenser,* detaching from the prootic somites, has plenty of large yolk-globules, whereas the ectomesenchyme has only a few small ones (Figs. 63, 67). The yolk remains even after the endomesenchyme has differentiated into morphological units. If the trabecles detach from the premandibular somites, their endomesenchymal cells should accordingly be expected to be

* As is well known the eggs of *Acipenser* have a holoblastic cleavage of a transitional type (cf. e.g. NELSEN 1953).

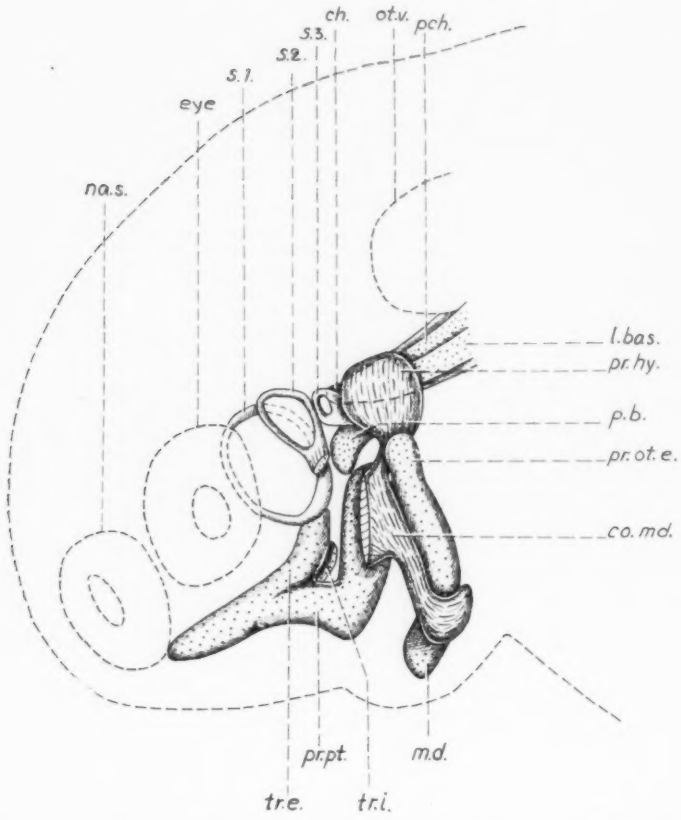

Fig. 68. *Acipenser ruthenus*, 8.6 mm. Reconstruction of anterior part of chondrocranium, lateral view. Indications as in Figs. 1—2. 100 ×.

initially laden with yolk. This is, however, not the case. Their cells have instead exactly the same histological appearance as those of the visceral skeleton (Fig. 67), that is, they are e c t o m e s e n c h y m a l.

Each trabecle is initially continuous with the tip of the palatoquadrate approximately in the medioventral part (Fig. 68). Caudally to this point the trabecle is formed in a medial and a lateral part (tr.e., tr.i.), separated by the carotis interna (c.i.) (cf. p. 291). Both parts are dorsally *connected* with the premandibular somite (s. 2) *but do not receive any cells from it* (Fig. 68) (cf. Hepsetus stage I—III).

HOLMGREN's suggestion that the posteromedial portion of the trabecles detach from the ventromedial part of the premandibular somites and the posterolateral portion from the ventrolateral "center" of the same somites (cf. HOLMGREN, Fig. 17) seems to be dubious. Actually, the cell-agglomerations in question instead

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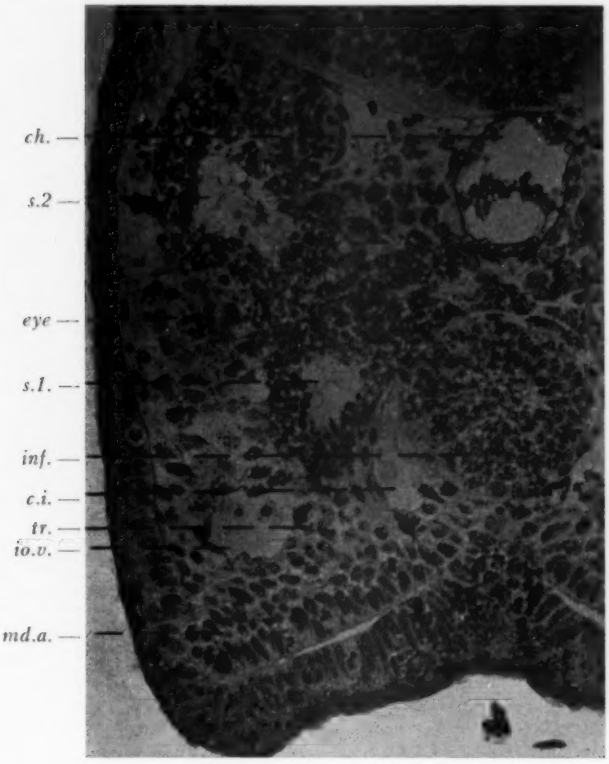

Fig. 69. *Neoceratodus forsteri,* 9.3 mm. Transversal section through left part of skull to show the position of the trabecle primordium. Mesoderm laden with yolk. 170 ×.

differentiate into the m.obliquus inferior and m.rectus inferior, respectively. However, since they insert on the trabecles it is very easy to get the erronous impression from the blastemal stage that the trabecles differentiate from the premandibular somites.

Also *Dipnoi* have holoblastically cleaving eggs of a transitional type (NELSEN 1953; and others) and the mesoderm and endomesenchyme are heavily loaded with yolk. Not in these fishes either do the yolk-globules of the endomesenchyme resorb until the elements have begun to form; in the parachordals they are left even after chondrification has set in. The ectomesenchymal cells, on the contrary, have only a few and small yolk-globules that distintegrate already at the blastema stage*. As in *Acipenser* there should hence be expected that if the posterior part of the trabecles detach from the premandibular somites their (endomensenchymal) cells should be loaded with yolk like the other endomesenchymal cells. This

* Also *Ambystoma* has such histological differences between endo- and ectomesenchyme (cf. STRASSER 1879; STÖHR 1879; LANDACRE 1921; DE BEER 1947; and others).

is not the case, however. As to the dipnoan trabecles it is accordingly important to state that also they are—as in *Acipenser*—ectomesenchymal all through.

In *Neoceratodus* the trabecle rudiments begin to differentiate at the 9.3 mm stage (Fig. 69). Each one is then situated between the premandibular somite, dorsally (s.l.), and the mandibular arch, ventrally (md.a.), and between the carotis interna, medially (c.i.), and the infraorbital vein, laterally (io.v.). Contrary to the conditions in acipenserids they are initially not in contact with the premandibular somites (Fig. 69). The mandibular arches are, by means of a blastema lamella, connected with the trabecles and later by means of a basal process (Fig. 51, pr.bas.) fused with the posterior part of the trabecles (cf. SEWERTZOFF 1902; HOLMGREN & STENSIÖ 1936; and others).—The trabecles are formed separately but soon (Fig. 50) connect caudally with the *endomesenchymal* polar cartilages.

Protopterus has essentially the same ontogeny and histology as *Neoceratodus* in this respect. Only late stages of *Lepidosiren* have been available. According to their general similarities with *Protopterus* there is, however, no reason to expect any differences as to the origin of the trabecles.

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The general conclusions concerning the origin of the trabecles may be summarized as follows.

It is shown that the trabecles in *Teleostei, Acipenser* and *Dipnoi* are caudally *bordered* by the ventral margin of the premandibular somites*. According to the different histology of endo- and ectomesenchyme, it has also been possible to determine that the trabecles are entirely ectomesenchymal in *Acipenser* and *Dipnoi*. Provided that the trabecles of the amphibians also are ectomesenchymal (cf. p. 297) and provided that they in *Agnatha* (p. 293) and *Selachii* (p. 293) really can be shown to be clearly dual in origin, there consequently seem to exist two types of trabecles: one type where the trabecles are formed only of ectomesenchyme (*Teleostomi, Dipnoi, Amphibia*) and one where they are formed of both endo- and ectomesenchyme (*Agnatha, Elasmobranchii*).

Were then originally the ectomesenchymal trabecles partly or entirely *visceral* elements, as ALLIS, DE BEER, JARVIK and others have suggested (p. 293)? This seems not à priori to be certain. HOLMGREN (1940) has shown that even other parts of the (selachian) neural cranium are ectomesenchymal (e.g. the medial parts of the taeniae marginalii, the dorsal and lateral parts of the otic cartilages, the antorbital cartilages and so on). These parts are very difficult to homologize with visceral elements. Further, a couple of rotation and transplantation experiments carried out by HÖRSTADIUS & SELLMAN (1946) show that the ectomesenchymal part of the trabecles (cf., however, p. 297) "cannot partake in the development of visceral arches" (HÖRSTADIUS 1950, p. 57), and, conversely, that pre-

* In *Neoceratodus* the trabecles are initially free from the premandibular somites but soon get in contact with them caudally.

sumptive branchial arch cartilage material does not produce trabecles.

These circumstances seem difficult to neglect in discussing the possible visceral origin of the trabecles. On the other hand it is obvious (1) that in *Dipnoi* the entirely ectomesenchymal trabecles caudally—as an infrapharyngomandibular element should be—are connected with the basal process of the mandibular arches, (2) that the conditions in the crossopterygian *Eusthenopteron* show that the (ectomesenchymal part of the) trabecles might be homologous with infrapharyngomandibular elements (JARVIK 1954), whereas other parts of the neural cranium might have incorporated infrapharyngeal elements of also the hyal and premandibular arches, and, finally, (3) that the present investigation of teleostomes and dipnoans have shown that there are even embryological support for the theory of a visceral origin of parts of the neural cranium (trabecles, lateral commissures) (chapter V). To settle this problem definitely, however, more embryological investigations are needed, experimental as well as comparative anatomical. At our present state of knowledge the collected embryological and paleontological evidences for a visceral origin of the trabecles may be considered more important.

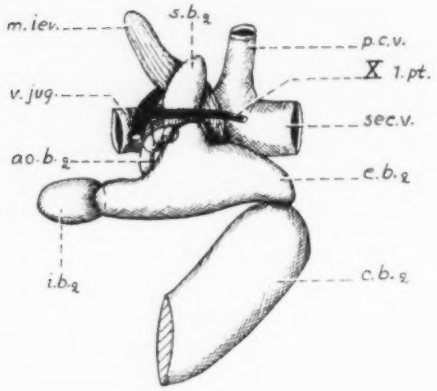
V. On the visceral chondro-cranium in fishes

MESOTIC ARCHE

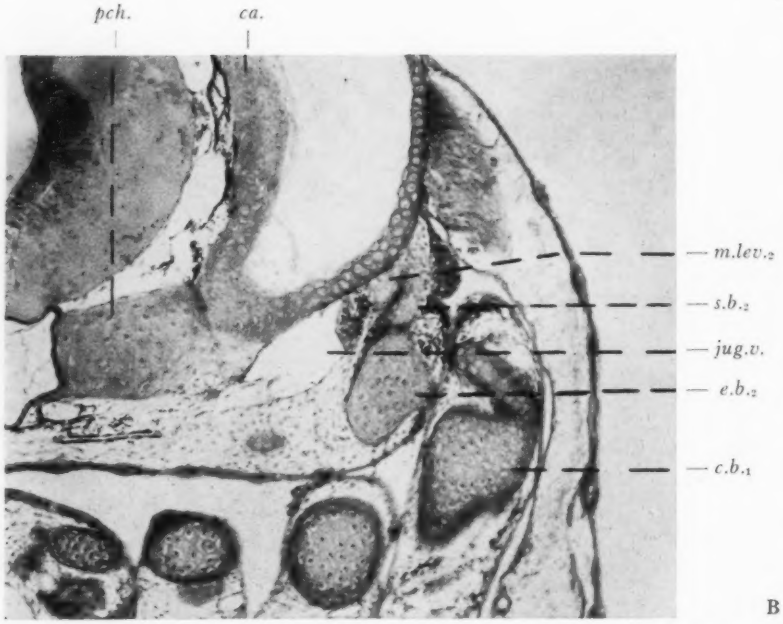
Any visceral theory concerning the prootic visceral arches in gnathostomian fishes must be based on studies of the mesotic visceral arches (the branchial arches).

Embryological and paleontological investigations have, especially in late years, given decisive evidence in support of the opinion that a normal ancestral teleostomian branchial arch consists not of four, as was earlier considered, but of five elements, a suprapharyngo-, infrapharyngo-, epi-, cerato- and hypobranchial. This is convincingly shown to be valid both for rhipidistian crossopterygians (JARVIK 1954) and paleoniscids (NIELSEN 1949). In recent teleostomes they seem to be best developed in the anterior branchial arches of *Acipenser* (DE BEER 1925; HOLMGREN 1943; and others). The infrapharyngeal element articulates with the ventromedial part of the otic capsule, and the suprapharyngeal element with the ventrolateral part of the capsule. Both elements are said to lie medially to the jugular vein (v.capitis lateralis) (cf., however, below), whereas the efferent branchial artery and the branchial nerve pass between them.

As to the ontogeny HOLMGREN describes how, in the first branchial arch of *Acipenser ruthenus,* the epibranchial of a 7 days embryo has a stout medial process below the efferent branchial artery (the whole arch is still blastemal) and how in a 25 day embryo this blastemal process has grown mediad and touches the basiotic lamina (the rest of the arch is chondrifying and dividing). At the 37 day stage it chondrifies independently of the epibranchial and forms a separate ele-



A



B

Fig. 70. *Acipenser ruthenus,* 14 mm. The position of the dorsal part of second branchial arch in relation to jugular vein. A, reconstruction, lateral view. 160 ×. B, transverse section on a level with the second suprapharyngobranchial. 160 ×.

ment, the infrapharyngobranchial. The suprapharyngobranchial simultaneously develops and gets in contact with the otic capsule more laterally (but still medially to the jugular vein). It chondrifies independently, too.

Here it should be added that a study of HOLMGREN's material (p. 205) reveals that also the suprapharyngeal element is continuous with the epibranchial in the blastema stage and that the same is the case even in the second branchial arch.

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Further is to be noted that the first as well as the second suprapharyngeal initially grow dorsad towards the jugular vein. The first suprapharyngobranchial then turns medially to the vein, while the second one instead turns *laterally* to the vein (Fig. 70). This is not mentioned by HOLMGREN or any other author and shows that the relation of the suprapharyngeal element to the jugular vein may vary.

Also in the recent crossopterygian *Latimeria chalumnae* (MILLOT & ANTHONY 1958) and in the actinopterygians *Polypterus* (VAN WIJHE 1882*; ALLIS 1918; PEHRSON 1947), *Amia* (HOLMGREN 1943) and *Lepisosteus* (HAMMARBERG 1937), the infrapharyngobranchials chondrify independently, the suprapharyngobranchials do not. The latter ones develop as a dorsal process of the epibranchials, caudally to the visceral nerve and aorta. This difference should, however, not be any major obstacle to their homologization with the independent suprapharyngobranchials in Acipenser, because, within one and the same species, e.g. *Eusthenopteron* (JARVIK 1954), the suprapharyngobranchial can exist either separately (the suprapharyngobranchial$_1$) or as a dorsal process of the epal element (suprapharyngobranchial$_2$).

Common to all teleostomian fishes mentioned above is both a tendency towards a reduction caudally of the pharyngeal elements and such an intimate connection with the epibranchials that they cannot be discerned any more. As to the suprapharyngobranchials this is probably in part due to their having no relations to the neurocranium.

Among recent selachians HOLMGREN (1940, 1942, 1943) has, as a result of comparisons with actinopterygians, homologized the ordinarily separate and posteriorly directed "pharyngobranchials" (or "infrapharyngobranchials" SEWERTZOFF 1897, 1899; VAN WIJHE 1904, 1922; and others) with suprapharyngeal elements. The interarcual cartilages are instead said to be the real infrapharyngobranchials. In acanthodians (WATSON 1937) the infra-pharyngobranchials were fully developed, and the small dorsocaudal process of the epibranchials may possibly have been a suprapharyngobranchial (HOLMGREN 1942).

Branchial arches forked dorsally may, finally, have been present also in cephalaspids (JARVIK 1954). In *Myxine* HOLMGREN (1946) found two undivided internal branchial arches parallel with the external ones. In *Petromyzon,* on the contrary, no internal branchial arches are described. As to the discussion of internal and external branchial arches compare e.g. JOHNELS (1948) and JARVIK (1954).

Of the gnathostomian fishes investigated by the present author *Hepsetus* and the other characids principally agree with *Latimeria, Polypterus, Amia* and *Lepisosteus* in having independent infrapharyngobranchials as well as suprapharyngobranchials developing as a dorso-caudal process of the epibranchials. Also the relations to nerves and vessels are the same. However, in *Latimeria* and the other

* VAN WIJHE named the two pharyngobranchials according to the position in relation to the efferent branchial artery.

fishes mentioned above, secondary fusions are described in the first—fourth branchial arches, and of the fifth branchial arch only a fused cerato-hypobranchial is present. In *Hepsetus* and the other characids, on the other hand, there are five comparatively complete branchial arches, in which only a fifth infrapharyngo- and hypobranchial are missing. Contrary to the other fishes a fifth episuprapharyngobranchial is hence formed in the characids investigated; it is, however, rather small. Among other teleosts GEGENBAUR (1878) has described a vestigial fifth epibranchial in *Clupea, Alosa* and *Alepocephalus,* and SAGEMEHL (1885) in the African characid *Citharinus.*

The genesis of the branchial arch skeleton in *Hepsetus* principally agrees with that of *Acipenser* as described by HOLMGREN (cf. p. 303). The exception is that the two first suprapharyngobranchials neither chondrify independently nor articulate with the otic capsules. The continuous slightly curved rods of dense blastema of the first stages (Figs. 1, 3—5), lying in close contact with the pharynx endoderm, later (Figs. 20, 30, 38) divide into the different elements mentioned above, simultanously with their chondrification. This type of ontogeny of the branchial arches was already described by VAN WIJHE (1904) and BRAUS (1905) in selachians and in actinopterygians e.g. by HOLMGREN (1943). The different elements hence do not form as the result of a secondary subdivision of a cartilage rod, a type of ontogeny that was originally described by DOHRN (1885/86) in selachians, PARKER (1882 a, b) in *Acipenser* and *Lepisosteus* and STÖHR (1882) in teleosts.

At the 7.3—8 mm stages the dorsal portion of each skeletal branchial arch (except for the fifth) forms a rostromedial process below the proximal part (the bend) of the efferent branchial artery. In the first arch it even gets in contact with the basis cranii. This blastemal process chondrifies separately at the 12.6 mm stage as the infrapharyngeal element (Fig. 31).

Another blastemal process of the dorsal end of each arch (inclusive the fifth) simultaneously grows dorsal caudomedially to the bend of the efferent branchial artery* (Figs. 21, 22). This is the suprapharyngeal element. It rises medially to the m.constrictor dorsalis but does not reach the jugular vein or the otic capsule as in *Acipenser.*

Finally, it remains to point out the interesting fact that the skeletal branchial arches in *Hepsetus* are initially connected with a densely nuclified dorsolateral blastema (Figs. 4, 5, l.br.$_{1-2}$) of the same type and position as the dorsolateral blastema in the prootic arches, i.e. the processus oticus externus in the mandibular arch (Fig. 4, pr.ot.e.) and the laterohyal in the hyal arch (l.hy.) (cf. p. 217). In the prootic as well at the mesotic arches this blastema is situated laterally to the visceral nerve, the visceral aorta and the constrictor dorsalis muscle and is connected with the epal element rostrally and caudally to these. It always consists of more irregularly arranged and smaller nuclei than those of the skeletal (inner)

* A fifth branchial artery does not exist.

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arch; they are also somewhat more stainable in Azan. In the prootic arches all or part of the dorsolateral blastema chondrifies in connection with the visceral skeleton, while the rest seems to dissolve or become connective tissue. In the branchial arches the whole dorsolateral blastema instead differentiates into dorsal gill rays (Fig. 35, g.r.).

From phylogenetical point of view it will hence seem as if certain of the prootic gill rays might have got other functions, when the gills of the prootic arches once were reduced or disappeared. The prootic gill rays then were able to persist only as secondarily transformed parts of the prootic visceral skeleton. This theory will be discussed from the basis of a comparative study of the literature and of a large actinopterygian and dipnoan material.

The dorsolateral blastema in the branchial arches in *Hepsetus* is ventrally continuous with a thinner subepidermal blastema of the same type, enclosing the visceral aorta and nerve from the outside. In the prootic arches this sheath may chondrify in connection with the skeletal arch (the lateral portion of the stylohyal), but most of it dissolves or becomes connective tissue. In the branchial arches it instead differentiates into gill rays belonging to the middle and ventral portion of the gills. For the moment there is only to add that the dorso-lateral blastema in the branchial arches is well developed also in other characids investigated but only present as diffuse vestigies in the other actinopterygians and the dipnoans studied by the present author. In them it only differentiates into connective tissue.

PROOTIC ARCHES

The prootic visceral arches in fishes are generally held to be two: the hyal and the mandibular arches. They are considered to be homologous with the mesotic visceral arches (the branchial arches) and to have been subdivided in the same manner as these. However, as to the identification of their various portions and if and to what extent their elements may have become incorporated into the neurocranium, there is considerable controversy between various authors (Allis 1897, 1915, 1918, 1923 b, 1925 a, 1928 a, b, 1931, 1938; de Beer 1924, 1926, 1931, 1937; Edgeworth 1923, 1926; Hofer 1945; Holmgren 1943; Jaekel 1899, 1906, 1925, 1927; Jarvik 1954; Romer 1937; Schmalhausen 1923; Sewertzoff 1916/17, 1923, 1931; Sewertzoff & Disler 1924; Säve-Söderbergh 1936; Watson 1951; and others).

During the past decades several theories have also been proposed concerning the possibility that vestigies of a premandibular arch are present in the gnathostomian skull. This problem has in late years been attacked from a new angle by Jarvik (1954).

Hyal arch

As to the hyal arch it is especially the hyomandibula, the symplectic and the stylohyal that through the years have attracted attention.

Hyomandibula

In his work "The Homologies of the Hyomandibula of the Gnathostome Fishes" ALLIS (1915) has reviewed the earlier literature on this problem, and in 1943 HOLMGREN in his work "Studies on the head of fishes" covered the literature through to the forties. The later literature is very scanty. Here only the more important trends shall be dealt with.

Selachii. Two different opinions seem to prevail as far as *sharks* are concerned. LUTHER (1909), ALLIS (1915) and DE BEER (1937) consider the hyomandibula to represent only an epihyal, the pharyngohyal being vestigial or absent. According to GEGENBAUR (1872), PARKER (1879), DOHRN (1884/85), SCHAUINSLAND (1903) and HOLMGREN (1943), however, the hyomandibula is an epi-pharyngohyal. HOLMGREN, who is the only of these authors making a clear distinction between supra- and infrapharyngeal elements, suggested more in detail that the pharyngeal element found in young embryos is a suprapharyngohyal. An infrapharyngohyal was said to be present (*Scyllium, Heterodontus*) in the shape of the lower post-spiracular ligament.

According to GEGENBAUR the hyomandibula of *rays* is homologous with that of sharks (an epi-pharyngohyal), whereas ALLIS considered it to be a pharyngohyal. However, before it was proved by KRIVETSKY (1917), DE BEER (1932), HOLMGREN (1940) and others that those parts, which had been considered since GEGENBAUR's days to be the epal and ceratal elements of the hyal arch, in reality represent the bases of hyal gill rays, fused with each other to a "pseudohyoid" (cf. Fig. 80), it was hardly possible to make a correct comparison between the hyomandibula of sharks and rays. DE BEER then homologized the hyomandibula of rays with that of sharks, suggesting it to be an epihyal. According to ALLIS (1915) and HOLMGREN on the other hand, the true hyomandibula in rays and sharks is differently composed: in rays ALLIS suggested it to be a pharyngohyal, whereas HOLMGREN found that the (supra)pharyngohyal fuses with the otic capsule, not with the epal element as in sharks; the infrapharyngohyal is missing*.

As to *Holocephalii,* finally, SCHAUINSLAND (1903), WOSKOBOINIKOFF (1914/15) and DE BEER & MOY-THOMAS (1935) have described independent epi- and (supra)pharyngohyals. However, in *Chimaera* there exists also, according to HOLMGREN & STENSIÖ (1936), a separate infrapharyngohyal. If that is the case, *Chimaera* is the only recent elasmobranch with a complete hyal arch. This ap-

* A separate "pharyngohyal" is illustrated by WOSKOBOINIKOFF (1914/15) in *Torpedo ocellata.* According to the position it seems, however, to be an interarcual cartilage of the first branchial arch.

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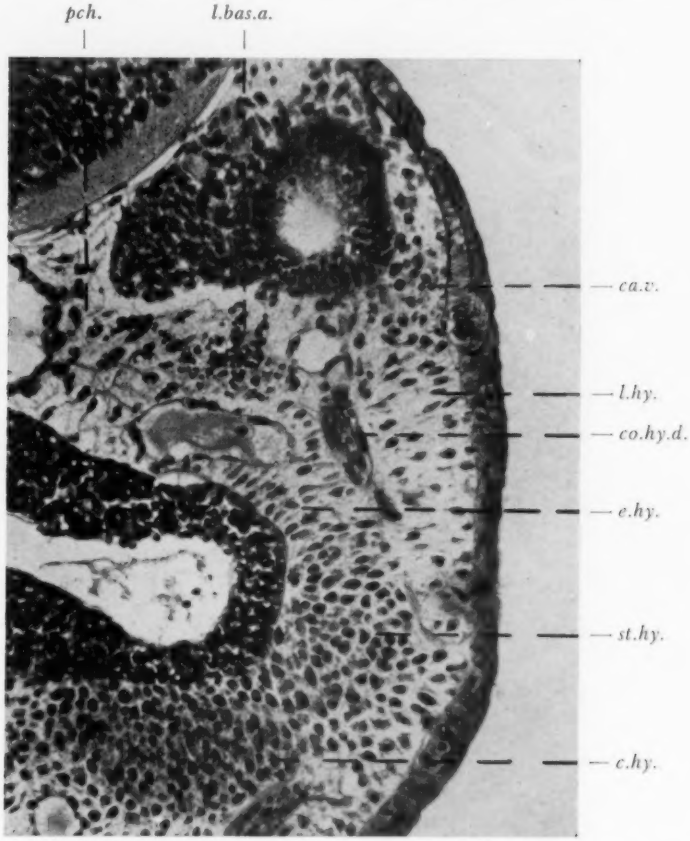

Fig. 71. *Neoceratodus forsteri,* 10.6 mm. Transverse section through right part of skull to show the differentiation and shape of hyal arch, parachordals and basiotic laminas. Endoderm and endomesenchyme laden with yolk. 100 ×.

parantly primitive nature of the hyal arch in the holocephalians is probably associated with the fact that it is non-suspensorial (cf. DE BEER 1937).

Dipnoi. Another group having autostyly (HUXLEY 1876; autosystyly DE BEER & MOY-THOMAS 1935) is the dipnoans. Of these fishes only *Neoceratodus* has the dorsal part of the hyal arch left (HUXLEY 1876, RIDEWOOD 1894; SEWERTZOFF 1902; KRAWETZ 1910; GREIL 1908—1913; ALLIS 1915; SCHMALHAUSEN 1923; DE BEER 1924, 1937; EDGEWORTH 1926; HOLMGREN & STENSIÖ 1936). All these authors, however, have only described one or two usually rather advanced stages, and none of them has compared the hyal arch with the branchial arches. Actually, HOLMGREN & STENSIÖ write that new investigations are badly needed.

Since an investigation of the hyal arch in *Neoceratodus* seems to be valuable for the general understanding of the homology of the hyomandibula, a description

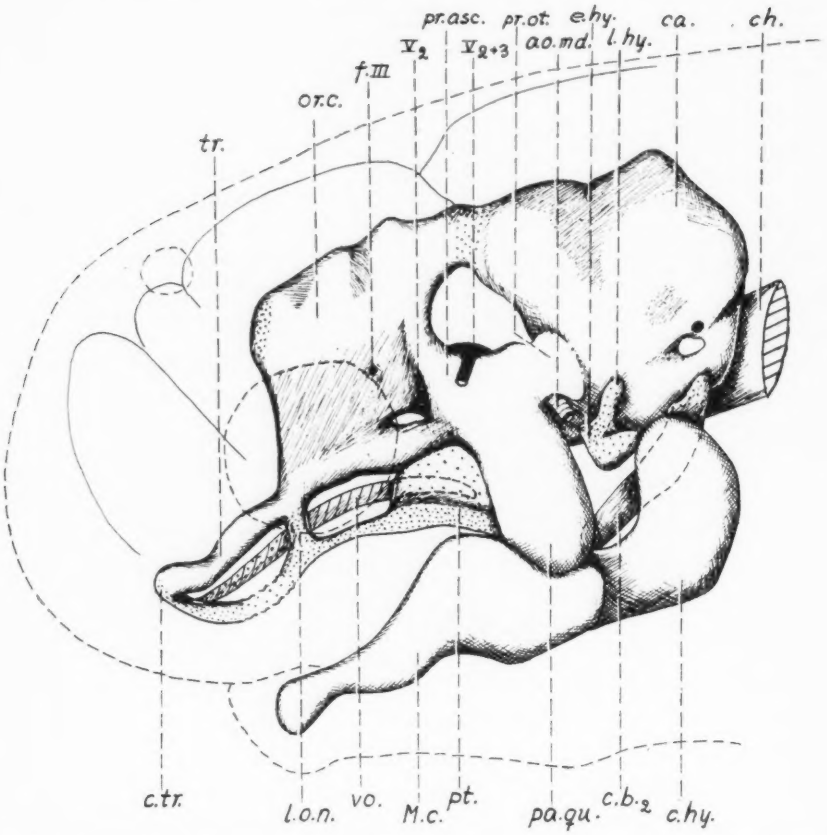

Fig. 72. *Neoceratodus forsteri,* 12.6 mm. Reconstruction of chondrocranium, lateral view. Indications as in Figs. 1—2, 13. 56 ×.

is presented in the following which gives some new results concerning the hyal arch in this fish. It is based on an extensive material (cf. p. 205).

In egg stages there is no essential difference between the hyal and branchial arches. They are each constituted by a core of mesoderm surrounded by a loose ectomesenchyme.

The part of the mesenchyme situated between the mesoderm and the pharynx endoderm condenses to form a skeletal arch at the 8.6 mm stage. The agglomeration of cells is most compact ventrally, where the future ceratal and hypal elements are situated. In the hyal arch the visceral aorta is also forming and the tr.hyomandibularis facialis is pushing its way downwards.

Later on the hyal arch dorsally becomes even more divided than the branchial arches, which dorsally only forms an epal element (Fig. 73). At the 10—10.6 mm stages a thick blastemal knob has been formed on top of the ceratohyal. It con-

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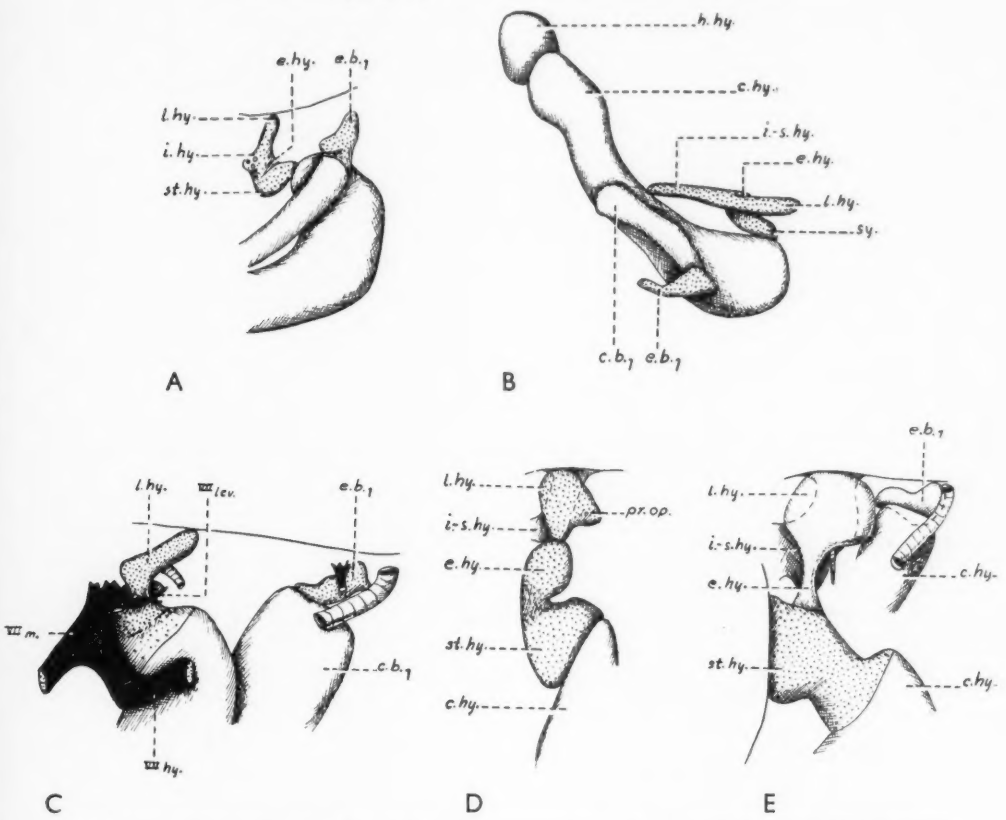

Fig. 73. *Neoceratodus forsteri,* 10.6 mm. Reconstruction of different stages in the ontogeny of the dorsal part of the hyal arch. The position of the first branchial arch is shown for comparison. Indications as in Figs. 1—2, 13. 93 ×. A, 12.6 mm, medial view. B, 12.6 mm, dorsal view. C, 14.5 mm, lateral view. D, 20 mm, lateral view. E, 24 mm, lateral view.

sists of concentrically arranged nuclei, derived from both the skeletal arch blastema, medially, and the subepidermal blastema, laterally, (Fig. 71). In comparison with actinopterygians (cf. e.g. *Hepsetus,* p. 216, Fig. 9) this knob seems to represent a stylohyal. This was already suggested by SCHMALHAUSEN*. The ventral part later becomes ligamentous (the hyosuspensorial ligament), whereas the dorsal part chondrifies. As RIDEWOOD has stated, there is a certain variation in the morphology of this and the other dorsal chondral elements of the hyal arch (Fig. 73). However, in the material investigated they are always discernable as separate entities.

The differentiation of the ventral part of the hyal arch proceeds rapidly. A separate hypohyal forms at the late blastema stage and at the 12.6 mm stage it

* HOLMGREN & STENSIÖ homologized it with an epihyal; no reasons for this are, however, given. EDGEWORTH's "interhyal" corresponds to the epihyal mentioned below.

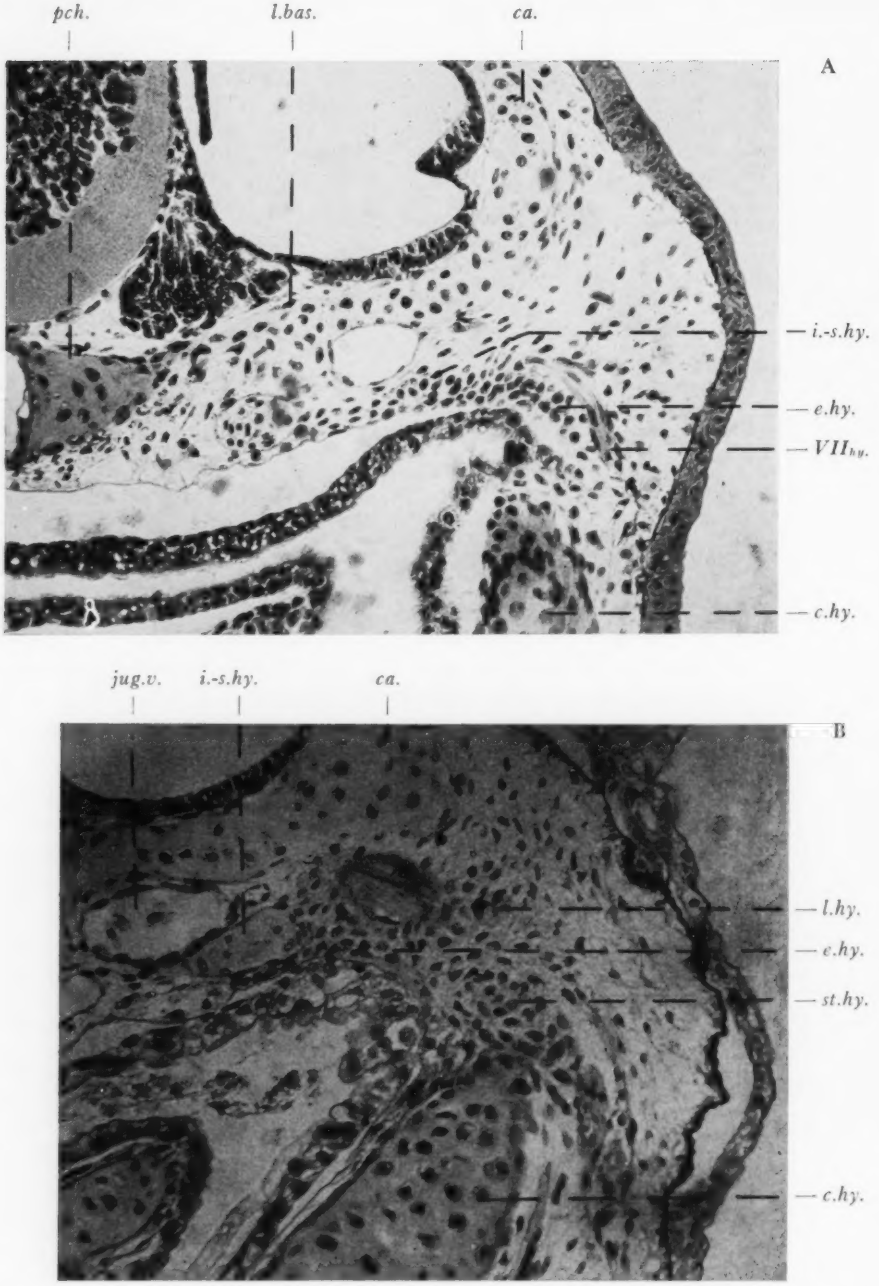

Fig. 74. *Neoceratodus forsteri.* Transverse section through right part of skull, on a level with the hyal arch. A, 12.6 mm. The lateral commissure (i.-s.hy.) has started to chondrify separately. 165 ×. B, 14.5 mm. The lateral commissure has chondrified and is detaching from the still blastemal epihyal and joining the neural chondrocranium laterally to the jugular vein. 160 ×.

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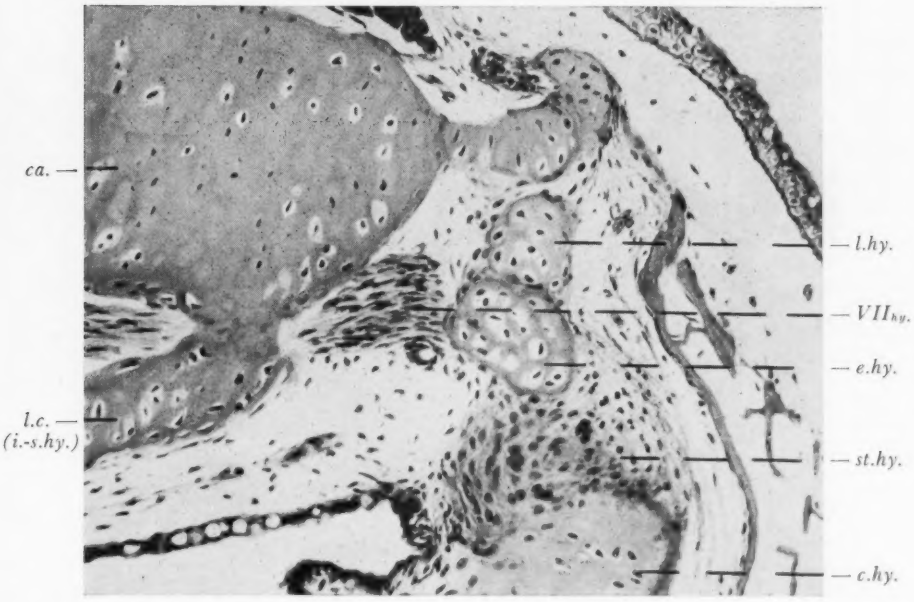

Fig. 75 *Neoceratodus forsteri*, 34.5 mm. Transverse section through right part of skull to show how the lateral commissure has joined the neural chondrocranium and how the stylo-, epi- and laterohyals may chondrify separately. 175 ×.

is beginning to chondrify as well as the ceratohyal. The dorsal portion of the arch is then still essentially blastemal (Fig. 72). Nevertheless a certain differentiation can be seen. Above the stylohyal the hyomandibular blastema has condensed into two elements: the ventral one is still blastemal (Fig. 74 A, e.hy.), whereas the dorsomedial (i.-s.hy.) has a small chondrification centre. If compared with the branchial arches the former portion has the position of an *epal* element*, whereas the chondrifying part** has no equivalent in the branchial arches. It is situated just ventrolaterally to the jugular vein and caudoventrally to the tr.hyomandibularis facialis and the orbital artery. Later this independently chondrifying portion of the hyomandibula separates from the more slowly chondrifying epal element (Figs. 74 A, B, 75). Instead it joins the basiotic lamina of the neurocranium. As it soon also connects laterally with the otic cartilage, there is in this way formed a lateral wall of the jugular canal (containing the jugular vein and the

* Greil and Edgeworth call both elements "epihyals"; otherwise it is only described by Goodrich (1930) and Schmalhausen, by the latter homologized with a symplectic. The epihyal later often becomes fused with the laterohyal. Lack of early embryonic stages may then explain the existing discrepancies in the literature.

** The "hyomandibula" Fürbringer and Krawetz, the "otoquadrate" Edgeworth and the "pharyngohyal" Schmalhausen. Holmgren & Stensiö could identify it but suggested it to be part of the neurocranial floor; their youngest embryo measured, however, 16 mm, a too advanced stage to give correct information of the origin of the element.

orbital artery) which seems to be homologous with the *lateral commissure* of teleostomes (cf. p. 328, Fig. 51).

It should be mentioned in this connection that ALLIS (1914b) has suggested that KRAWETZ' "hyomandibula" constitutes one part of the lateral commissure in *Neoceratodus*. The other part of the commissure might be the "pars ascendens" of the palatoquadrate (GREIL's collective term for processus ascendens and processus oticus), homologous with the fused pharyngeal elements of the premandibular and mandibular arches. The former statement is only partly acceptable, as KRAWETZ' "hyomandibula" is the whole lateral commissure according to the opinion of the present author (cf. p. 313, note 2). The other statement of ALLIS seems on the contrary to be quite a hypothetical one and not founded on investigations of his own or even on sufficient information from other authors. Actually the "pars ascendens" is, judging from its ontogeny, a portion of only the mandibular arch and it will not even secondarily fuse with the lateral commissure. Further, it is situated anterior to the spiracle and anterolaterally to the mandibular aorta (Fig. 72, ao.md.), a position unknown to the lateral commissure in teleostomes. In these fishes the lateral commissure is instead situated posterior to the spiracle and caudomedially to the mandibular aorta*.

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When considering the homology of the lateral commissure in fishes JARVIK (1954) has suggested the lateral commissure in Eusthenopteron to belong to the hyal arch and represent a suprapharyngeal element. The present investigation of *Neoceratodus* strengthens the theory of a hyal origin of the lateral commissure, but exactly what element it represents is hard to determine, because the dipnoans have no pharyngobranchials to compare with. If compared with the teleostomian branchial arches, however, the first branchial arch of e.g. *Eusthenopteron* has a basally fused infra-suprapharyngeal element and the visceral nerve runs down anterior to it. The angular lateral commissure in *Neoceratodus* has essentially the same shape and relation to the skull and the visceral nerve, and therefore it might be homologous with a fused infra-suprapharyngohyal. If that is the case, *Neoceratodus* has initially a complete hyal arch principally comparable with that in *Chimaera* (p. 308).

There is, finally, another element left to describe in the hyal arch in *Neoceratodus*. It appears at the 10.6 mm stage (Fig. 71, l.hy.), but constitutes a rest of the dorsal portion of the subepidermal mesenchyme. This rest is by then polynuclear and situated outside the anterior part of the m.constrictor dorsalis. It is connected with the dorsolateral part of the epihyal. All these facts indicate that it corresponds to the laterohyal blastema in *Hepsetus* (p. 216). It is, however, smaller than in *Hepsetus*, situated between the tr.hyomandibularis facialis, rost-

* HOLMGREN (1943) has homologized the teleostomian lateral commissure with processus oticus internus of the selachian mandibular arch; this theory, however, seems improbable and does not tally with the results of the present or other authors (cf. p. 339).

rally, and the hyal aorta caudally (Fig. 73 C). It reaches the otic cartilage, dorsally, and chondrifies very slowly.

In the 14.5—20 mm specimens (Fig. 73) the laterohyal grows backwards and broadens. The posterior end projects towards the operculum; this part of the laterohyal hence corresponds to the processus opercularis in teleostomes. According to HOLMGREN & STENSIÖ this process in *Neoceratodus* can become independent. This agrees with the results of the present investigation.

In a 24 mm specimen (Fig. 73) the laterohyal has certainly chondrified in connection with the epihyal forming a club-shaped element, the hyomandibula, in which the epihyal constitutes the shaft. This is the adult shape of the "hyomandibula" of HUXLEY* (cf. HOLMGREN & STENSIÖ's Fig. 291 of a 27 mm embryo). In a 34.5 mm specimen, however, the epihyal and the laterohyal have on the contrary chondrified separately (Fig. 75). If phylogenetically considered, the separate formation of the epi- and laterohyal (cf. above and Fig. 71), their different shape (Fig. 73) and occasional separate chondrification (Fig. 75) seems to indicate a primarily separate origin of the two elements.

In the branchial arches the dorsal portion is an epal element, pointing dorsocaudad. The relation to the branchial nerve and aorta is seen in Fig. 73. The dorsolateral blastema is only vestigial, since no gill lamellae differentiate on the dorsal parts of the epibranchials.

Teleostomi. Contrary to the case in selachians the teleostomian hyomandibula articulates with the otic capsule laterally to the jugular vein. To explain why they do so, why the higher teleostomian hyomandibula is pierced by tr.hyomandibularis facialis and why it articulates by means of two articular heads, several theories have been proposed. Many of them, however, are rather dubious. As they are commented on by ALLIS (1915) and HOLMGREN (1943), only the more important theories will briefly be delt with here.

Beginning with the hyomandibular morphology, it can be noted that in recent lower actinopterygians, no canal is formed for the tr.hyomandibularis facialis (cf. e.g. VAN WIJHE 1879—1882 and SEWERTZOFF 1928). This is also the case in certain palaeoniscids, whereas in other palaeoniscids the hyomandibula is pierced by a canal for at least a branch of the tr.hyomandibularis. This canal is differently placed in different genera. It lies near the anterior border of the hyomandibula in *Broughia* (STENSIÖ 1932) or near the caudal margin for instance in *Australosomus* (NIELSEN 1949); here it pierces just anteroventrally to the base of the opercular process. According to ALDINGER (1937) there is instead in *Pygopterus* and *Acropholis* a shallow groove on the caudal margin of the hyomandibula. However, "disregarding the behaviour of the hyomandibular nerve," HOLMGREN writes (lc., p. 98), "the hyomandibula of *Polypterus* and of the *Palaeoniscids* is built as that (of bony Ganoids and) of Teleosts ... The conclusion must be that the

* Hyomandibula HUXLEY, VAN WIJHE, RIDEWOOD, SEWERTZOFF and SCHMALHAUSEN; symplectic KRAWETZ; interhyal EDGEWORTH; Zwischenstrang GREIL.

position of the hyomandibular nerves and its branches is variable." Why that is the case, HOLMGREN has not tried to explain*.

An attempt to interpret the course of the tr.hyomandibularis in teleostomes is, however, made by EDGEWORTH (1911). He described the ontogeny of the hyomandibula, inferring that it develops in the same way in *Acipenser* and *Polypterus*** and principally also in teleosts and bony ganoids, though "the VIIth nerve, at first winding round the hyoid bar, subsequently pierces the hyomandibula owing to chondrification spreading around it." This statement is criticized by HOLMGREN, who could not find that the hyomandibula is initially situated anterior to the nerve and then grows backwards, enveloping it. Also the present investigation supports this objection but has also shown one exception, viz. *Lepisosteus* (cf. p. 320).

ALLIS (1915) has suggested another explanation, viz. that the hyomandibula in most teleostomes is the result of the fusion of several structures. He did not, however, make any embryonic investigations of his own to support this theory but founded it partly on EDGEWORTH's description and partly on the conditions found by KRAWETZ in *Neoceratodus*. Because of these facts as well as an investigation of the adult *Torpedo*, ALLIS suggested that the anterior articular head of the teleostean hyomandibula is "an interarcual cartilage, which has fused with the pharyngeal element of the hyal arch"—in which the stylohyal is said to represent the epihyal—"and the posterior articular head is the extrabranchial of the arch fused with the same element." However, no reasons what so ever have been given for the latter homology. Against it may also be said that recent teleostomian branchial arches do not have any extrabranchials*** (HOLMGREN 1943)—and probably no interarcual cartilages either.

In 1918 ALLIS altered his opinion. The teleostean anterior articular head was now said to represent an "anterior branchial-ray bar of the hyal arch"****. The reason was that BRAUS (1905) had considered the selachian ray bars to belong to an independent category of skeletal elements, lying at right angles to the rays and parallel to the internal branchial bars. ALLIS therefore concluded: "These relations to the branchial-rays at once suggest a branchial ray bar" to have been present even in the hyal arch, i.e. if the posterior articular head still represents an extrabranchial (cf. ALLIS 1915) the anterior articular head should correspond to a ray bar.

* To this may be added that also in *Gadus* (DE BEER 1937) and *Cyclopterus* (UHLMANN 1921) tr.hyomandibularis does not pierce the hyomandibula. This element has, however, otherwise essentially the same morphology as that of other teleosts, and therefore their homology is quite probable. If so, the nerve must be the variable element also here.

** Making no account, however, of the fact that the relation to rr.hyoideus and mandibularis of the tr.hyomandibularis is not the same in both (cf. VAN WIJHE 1882).

*** "The extrabranchials are chondrified elements, considered to be specially modified dorsal and ventral branchial rays" (HOLMGREN 1943, p. 48). EL-TOUBI (1952) has shown this more in detail (p. 326).

**** A ray bar is the fused bases of the gill rays (cf. ALLIS 1918/19; HOLMGREN 1943; and others).

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DE BEER (1924, 1925, 1937) agreed with ALLIS' theory but instead started from the "familiar fact that the mandibular arch frequently has two or more articulations with the neurocranium". The "processus palatobasalis" is suggested to be the original top of the arch, lying medially to the jugular vein and the nerves. The other top, processus oticus, articulates with the skull in a more lateral position, lying laterally to the vein and the nerves. As to their origin, processus oticus is said to be (adopting ALLIS' suggestion) a modified ray bar.—Now DE BEER asks whether the hyal arch originally may not also have had two articulations, of which recent selachians have the basal and *Acipenser* the otic one. The articular head in *Acipenser* should hence represent a modified ray bar (1925). In *Holostei* "another branchial ray bar" is "plastered" on to the hyomandibula from the back, constituting the posterior articular head and enclosing the tr.hyomandibularis between the two bars. As an intermediate primitive type between the selachian and teleostomian types DE BEER in his first paper (1924) points to *Neoceratodus* and its forked hyal arch as it was described by SCHMALHAUSEN. In 1925, however, DE BEER started from quite a hypothetical type.

However, none of these theories concerning the morphology of the hyomandibula were founded on enough embryonic material and therefore seem too constructive. They were also more or less refuted by HOLMGREN (1940, 1942, 1943), who in his investigations laid the stress upon the earlier embryonic stages and on the fact that the relationship of the nerves, vessels, ligaments and muscles of course are important but may not be regarded as fixed once and for all. He studied the ontogeny of the hyomandibula in different actinopterygians (*Acipenser, Amia, Lepisosteus* and *Salmo*) and reached the opinion that it is principally built in the same way. Ventrally is an epal element, and on top of this a suprapharyngeal element is attached (the anterior articular head in *Amia, Lepisosteus* and *Salmo;* the dorsal articular disc in *Acipenser*). On the caudal margin of the latter he deduced there had been in holosteans and teleosteans "a ray bar, consisting of a dorsal ray, forming a posterior articular head and a ventral portion with the opercular process. In *Lepidosteus* in addition a subopercular ray (subopercular process) is present" (HOLMGREN 1943).

Referring to ROMER's (1941) account of *Megalichthys* HOLMGREN also suggested that the crossopterygians had a hyomandibula "probably the same as in Palaeoniscids, thus consisting of an epihyal (+ pharyngohyal?) and a ray bar on its caudal border". JARVIK (1954) has on the contrary—according to the regular disposition of the dental plates and the serial arrangement of the branchial arches—suggested the hyomandibula in *Eusthenopteron* to be entirely an epal element, the pharyngeal ones having fused with the neurocranium (the suprapharyngeal = the lateral commissure; the infrapharyngeal = the otic shelf in selachians).—As to the coelacanthids, finally, MILLOT & ANTHONY (1958) have described in *Latimeria* a hyomandibula, dorsally consisting of a broad cartilaginous plate, pierced by the tr.hyomandibularis facialis and called a pharyn-

gohyal, and a ventral ossified shaft, the epihyal. The latter is also found by STENSIÖ (1922) in *Wimania*. The separation between these elements, however, is probably not a primitive character but secondarily brought about by the retention in cartilage of the dorsal part during the ossification process. The hyomandibula in *Latimeria* should accordingly be homologous with that of other crossopterygians and the actinopterygians (cf. below).

In *Agnatha* HOLMGREN (1946) described an undivided internal hyal arch in *Myxine* and JOHNELS (1948) suggested such an arch to be present also in *Petromyzon*.

Very little is known about the early development of the hyomandibula in *Teleostei*. As a matter of fact only *Salmo salar* (HOLMGREN 1943) has been investigated with such an extensive material that it has yielded adequate results (cf. Fig. 76). It is therefore of great interest to see if the ontogeny of the characidean hyomandibula is comparable to that of *Salmo*, and if there are any bearings on the general problem of what the hyomandibula really is.

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As was shown in chapter III and in Fig. 1 the hyomandibula in *Hepsetus* is formed immediately behind the spiracular vestige. It primarily consists of two parts, a ventromedial (the future epal element of the inner arch, if compared with the branchial arches) following the curve of the pharynx endoderm, and a subepidermal, situated dorsolaterally to the other and laterally to the m.constrictor dorsalis (Fig. 4). These two rudiments do not consist of the same type of blastema: the epihyal and the rest of the inner arch has larger, more regularly arranged nuclei (cf. p. 306), whereas the dorsolateral blastema, the laterohyal, has smaller, irregularly arranged nuclei, more easily stainable in Azan. The border between them is further accentuated by the tr.hyomandibularis facialis, the efferent hyal artery and the m.constrictor hyalis dorsalis (Fig. 4). As was shown in chapter III and also pointed out on pp. 306—307 the dorsal portions of the branchial arches as well as of the mandibular arch are principally formed in the same way. It is therefore very tempting to homologize the laterohyal with transformed hyal gill rays (cf. p. 307).

At the 7.3 mm stage (Fig. 5) the laterohyal is dorsally in contact with the anterior otic cartilage, forming an anterior and posterior articular head and, caudally, an opercular process. The chondrification sets in at the 8 mm stages (Figs. 12, 15), but the structural boundary between the two parts can, in the beginning, still be discerned on each side of the foramen hyomandibularis. The efferent hyal artery is reduced at these stages; hence the adult hyomandibula (Fig. 36) is pierced only by the tr.hyomandibularis facialis. The m.constrictor dorsalis simultaneously differentiates into the m.dilator opercularis and the m.adductor hyomandibulae (medially).

Comparing with *Salmo* (Fig. 76) as described by HOLMGREN, *Hepsetus* shows essentially the same ontogeny of the hyomandibula. It is also of special interest that HOLMGREN points to the primary difference in the arrangement of the cells

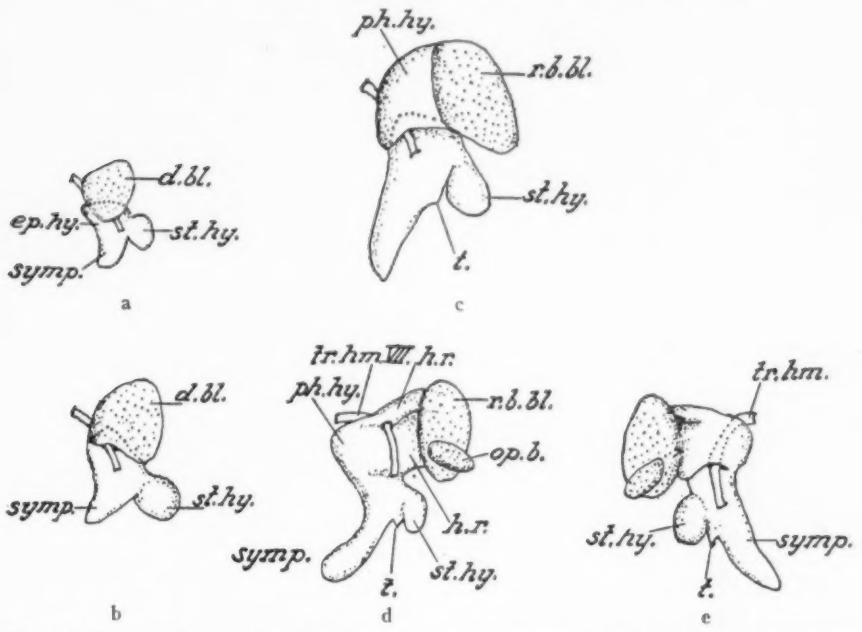

Fig. 76. *Salmo salar*. A series of reconstructions of the hyomandibula, lateral view; a, embryo 20 days 5 hours; b, 22 days 7.5 hours; c, 25 days 6.5 hours; d, and e, 26 days 1.5 hours, left and right hyomandibula. From HOLMGREN (1943, Fig. 44).

of the two parts (the inner hyal arch and the laterohyal). The difference between the two species is that in *Salmo* the tr.hyomandibularis facialis varies to a certain degree in relation to the hyomandibula: sometimes—as in *Hepsetus*—it runs between the inner arch and the laterohyal, sometimes it pierces only the latter part. This variation may be compared with that in palaeoniscids (p. 315), and is phylogenetically explained by the theory that the laterohyal represents fused gill rays (cf. above).

Furthermore, HOLMGREN has illustrated (but not described) how the "dorsal blastema" (the laterohyal) (Fig. 76 a), which is separate and single in the beginning, later divides into an anterior "suprapharyngeal" and a posterior "ray bar" blastema (Fig. 76 c). The former later differentiates into the anterior articular head and the latter into the posterior articular head, the opercular process and the opercular bone (Fig. 76 d). However, no such division of the laterohyal is seen in *Hepsetus,* and the present author has not been able to find it in *Salmo salar* either (HOLMGREN's material). The division may accordingly be hypothetical. If so, not only the posterior articular head and the opercular process, as HOLMGREN suggested, but also—as in *Hepsetus,* cf. p. 307—the anterior articular head should represent transformed gill rays in *Salmo.*

Besides, it has been shown in this investigation that the suprapharyngobranchials in *Acipenser* (p. 304) and *Hepsetus* (p. 306) develop from the epibranchials and not separately. Further, each one shows the same histological type as the rest of the inner arch.* But according to HOLMGREN the "dorsal blastema" of the hyomandibula in *Salmo* (the laterohyal) is formed *separately* and has *another* histological type than the inner arch (cf. above). There are then reasons to believe that the interpretation given above is correct: the laterohyal ("dorsal blastema") does not represent, all or partly, a suprapharyngohyal in *Salmo* either.

According to HOLMGREN the ontogeny of the hyomandibula in *Salmo* agrees fairly well with that of *Amia* and *Lepisosteus*. If this is true and if the theory set forth by him is correct, *Amia* and *Lepisosteus* should then have, in contrast to *Hepsetus* and *Salmo,* a laterohyal divided into an anterior "suprapharyngeal" and a posterior "ray bar" portion. In order to see if there really is a difference between them, the present author has reinvestigated HOLMGREN's material and has in addition studied some earlier stages of *Lepisosteus,* not described by HOLMGREN. As *Amia* agrees with *Lepisosteus* in this respect (cf. HOLMGREN), only the latter is described.

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The youngest specimen used by HAMMARBERG (1937) and HOLMGREN is 9.3 mm long. This is illustrated by HAMMARBERG, but as he took no notice of the blastema, it is pictured in this paper (Fig. 77 B). As in *Hepsetus* a continuous laterohyal can be seen; ventrally, however, it is connected with the inner skeletal arch only anteriorly to the r.hyomandibularis facialis (VII.hy.).

The laterohyal of this 9.3 mm stage of *Lepisosteus* hence has partially another morphology than in *Hepsetus* and *Salmo*. If a younger embryo is investigated, however, no difference can be seen. In e.g. a 8 mm specimen (Fig. 77 A) the laterohyal is also posteriorly connected with the inner skeletal arch. If compared with HOLMGREN's descriptions of *Amia* this stage corresponds to a 7 mm stage of the latter species. At the 11 mm stage (HAMMARBERG Fig. 3) the tr.hyomandibularis has again become enveloped by the laterohyal, and at the 12 mm stage an opercular process is forming caudally and a subopercular process caudoventrally. The latter chondrifies separately at the 18.7 mm stage. HAMMARBERG suggested the latter to be a hyal gill ray; HOLMGREN agreed and added that the opercular process is probably also a gill ray, whereas the rest of the hyomandibula represents an epi-infrapharyngohyal, ventrally, and a suprapharyngohyal, dorsally.

This investigation has shown that also *Lepisosteus* and *Amia* has a continuous laterohyal of principally the same type and morphology as that of *Salmo* and *Hepsetus*. Judging from the conditions in *Hepsetus* it is therefore possible to agree with HAMMARBERG's and HOLMGREN's suggestions of a gill ray origin of the opercular and subopercular processes. It is to be added, however, that also

* No suprapharyngobranchials are described in *Salmo,* but they seem to be represented by a dorsal process on the epal elements as in *Hepsetus*.

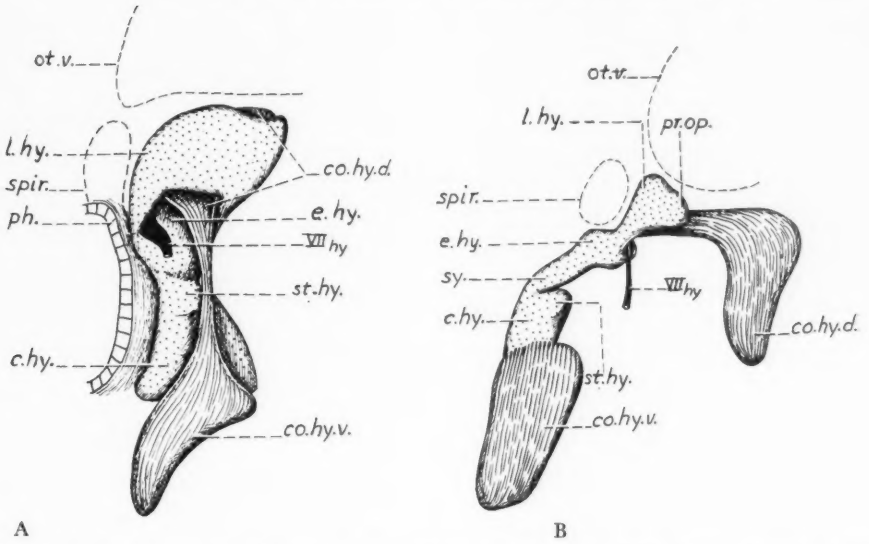

Fig. 77. *Lepisosteus platystomus*. Reconstruction of hyal arch, lateral view. Indications as in Figs. 1—2. 93 ×. A, 8 mm. B, 9.3 mm.

the rest of the laterohyal should represent transformed gill rays (and not a suprapharyngohyal).

These findings have made the present author also take into consideration HOLMGREN's other representatives for the actinopterygians: *Acipenser* and *Polypterus*. It has been found then that also in *Acipenser* a laterohyal is primarily present as in teleosts and holosts (Fig. 78). This is already described by HOLMGREN (1943): "Outside the upper part of the rudiment of the hyomandibula there lies a rather scattered mesenchyme, which dorsally enters between the upper end of the rudiment and the blastematic auditory capsule. In this mesenchyme at the 9 mm stage of *Acipenser ruthenus* a denser portion has arisen* ... Connected with this dorsal blastema is a blastematic strand (HOLMGREN 1943 Fig. 41, op.pr.) extending for some distance along the anterolateral part of the hyomandibula. This strand, probably representing a ray-bar**, detaches from the dorsal blastema and forms the rudiment of the small process (opercular process) of the hyomandibula" (HOLMGREN 1943, p. 84). In *A.güldenstaedti* this process is chondrified and bridges over the rr.mandibularis and hyomandibularis (HOLMGREN & STENSIÖ 1936).

The further ontogeny of the laterohyal in *Acipenser* deviates from the holostean-teleostean type described above. HOLMGREN (1943) described how the upper portion of the laterohyal ("dorsal blastema") after having separated into two

* = The suprapharyngohyal according to HOLMGREN; the stage is illustrated in Fig 41, HOLMGREN 1943.

** Cf. BRIDGE 1897, ALLIS 1915, see also p. 317.

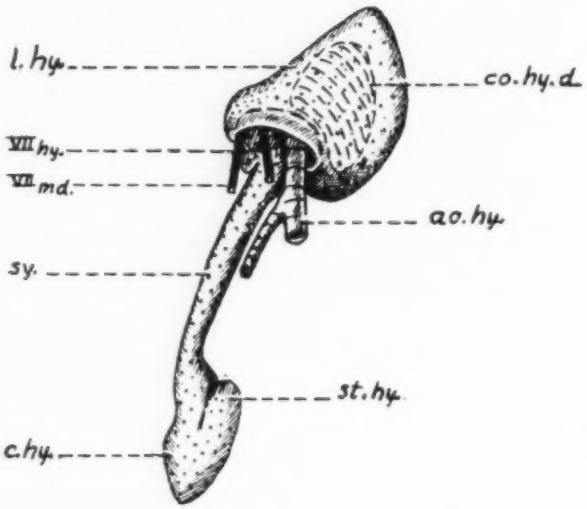

Fig. 78. *Acipenser güldenstaedti*, 9 mm. Reconstruction of hyal arch, lateral view. Indications as in Figs. 1—2. 172 ×.

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parts, "one part of which is probably fused on to the hyomandibula and another on to the otic capsule, later becomes flattened out and finally the only visible remainder of it forms the thin articular disc on to which the hyomandibula articulates." As to the rest of the laterohyal, the "blastematic strand" (cf. above), it "represents the opercular process and may be regarded a ray bar more or less strongly fusing on to the epihyal." If considering the laterohyal as a remnant of hyal gill rays (cf. p. 307), no objections can be raised against this homologization (Fig. 81).

Finally, very little is known about the earliest development of the hyomandibula in *Polypterus*. Moy-Thomas (1933) illustrated a very rudimentary hyosymplectic which at the 8 mm stage was chondrified and at the 9.3 mm stage dorsally forms a dorsal articular head and a posterior opercular process. The two latter stages were reinvestigated by Edgeworth. As his reconstructions, however, are in very slight agreement with those of Moy-Thomas, and as the ontogeny of the branchial arches are not completely known, a new investigation of this material is highly needed (cf. Holmgren 1943, p. 88).

The 6.75 mm stage is horizontally sectioned and badly preserved, not permitting any safe conclusions as to the origin of the hyomandibula. The 8 mm stage, however, gives a rather good idea of it (cf. the reconstruction in Fig. 79). The hyomandibula consists of young chondrocytes and has a rod-like shape, as Moy-Thomas stated. On the top of the hyomandibula a blastemal cord (Fig. 79, l.hy.) extends rostrocaudad from the spiracle. Rostrally it has a subepidermal position free from the hyomandibula, whereas the posterior end lies in contact with the hyomandibula, ventrally, and with the otic capsule, medially. This blastema is not described before. It has a special interest here, as it according to its position

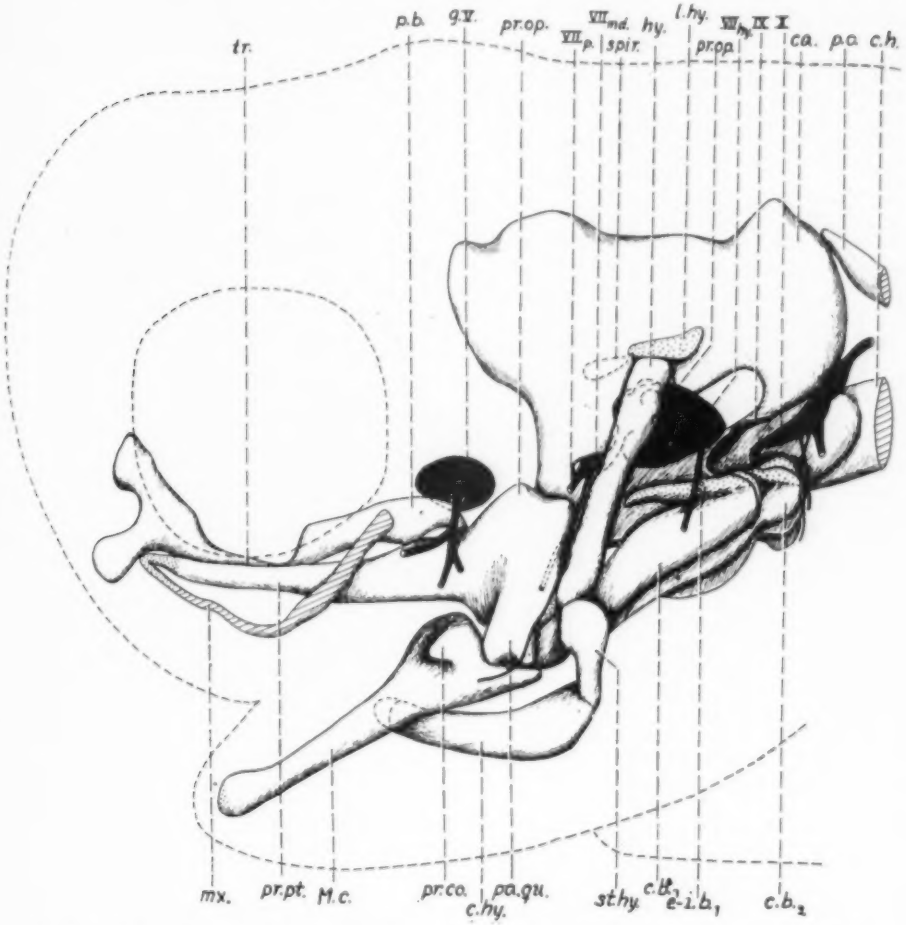

Fig. 79. *Polypterus senegalus*, 8 mm. Reconstruction of chondrocranium, lateral view. Indications as in Figs. 1—2, 18. 110 ×.

dorsolaterally to the hyomandibula seems to represent a vestigial laterohyal. No other traces of a laterohyal blastema is seen but may be present at earlier stages, as an opercular process is already jutting out caudally (pr.op.).—The two anterior branchial arches have, dorsally, a long, slender blastemal process pointing rostrad (Fig. 79, e-i.b.$_1$). The first arch has, in this process, an independent chondrification centre, representing the first rudiment of a separate infrapharyngeal element. No dorsolateral blastema is seen in the branchial arches.

Because of lack of stages it is hard to follow step by step the fate of the laterohyal blastema. It seems, however, as if, at the 9.3 mm and 9.6 mm stages (the latter does not belong to Moy-Thomas' material, cf. p. 273), it has fused with the

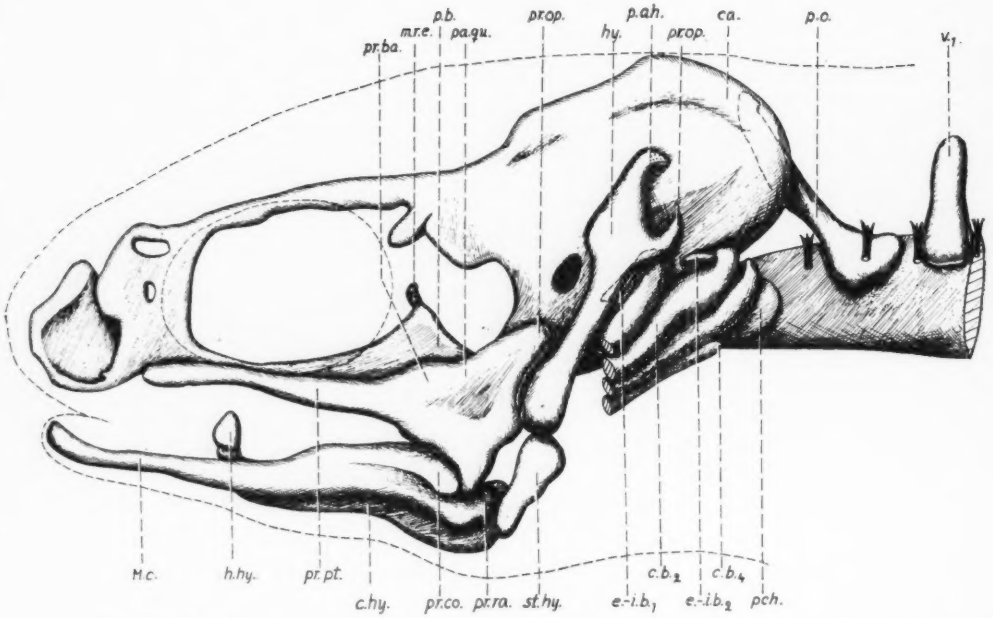

Fig. 80. *Polypterus senegalus,* 9.6 mm. Reconstruction of chondrocranium, lateral view. 60 ×.

top of the hyomandibula, forming the posterior articular head; the posterior tip of this is still blastemal (Fig. 80, p.ah.). On the caudal margin of the hyomandibula a large opercular process has now developed.

This investigation has shown that also in *Polypterus* there are reasons to believe that the essential part of the hyomandibula is an epal element (Fig. 81), on top of which a laterohyal fuses, forming at least the posterior articular head and probably also the opercular process. If compared with the other actinopterygians investigated these structures should hence represent transformed gill rays also in *Polypterus.*

ALLIS (1918, 1922) came to the same conclusion based on other reasons. He found that the accessory hyomandibula* contains a nodule of cartilage, which probably "represents the dorsolateral end of the bar formed by the fusion of the bases of the posterior row of branchial rays of the hyal arch, here not completely differentiated to form a posterior articular head of the hyomandibula such as is found in the *Teleostei*". This theory was principally adopted by HOLMGREN (1943).

In short this comparative survey of the *development* of the actinopterygian

* The term was introduced by TRAQUAIR (1871) and discussed by e.g. PARKER (1873), VAN WIJHE (1879—1882), ALLIS (1918, 1922), NIELSEN (1936, 1949), HOLMGREN (1943) and JARVIK (1954).

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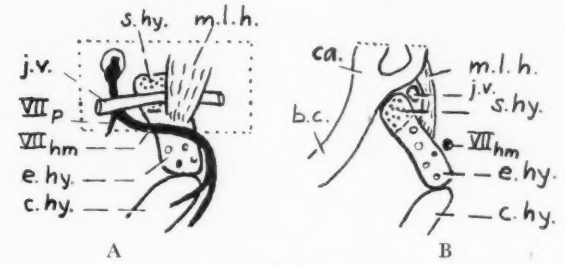
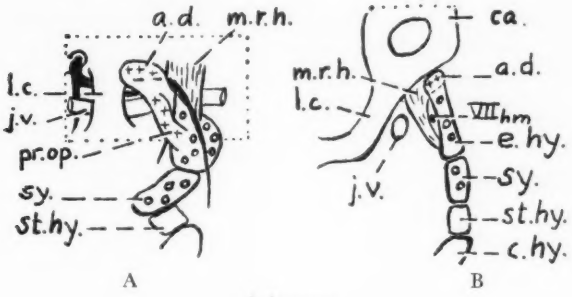
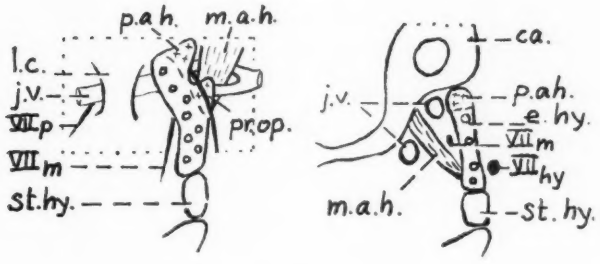


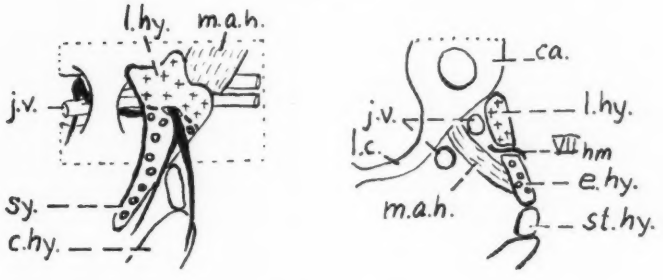
Selachii



Acipenser



Polypterus



Holostei, Teleostei

Fig. 81. Diagrams illustrating structure of hyomandibula and its relation to skull, nerves, veins and muscles in *Pisces*. A, lateral view; B, transverse section. Indications as in Fig. 85.

hyomandibula has shown (cf. Fig. 81): (1) A continuous dorsolateral blastema of the hyal arch, a laterohyal, forms in *Dipnoi** and *Teleostomi.* (2) In *Teleostei, Holostei* and probably *Crossopterygii* the laterohyal is large and develops the anterior and posterior articular heads as well as the opercular and (in *Lepisosteus*) the subopercular processes. (3) In *Chondrostei* the laterohyal is rather small and only forms the dorsal articular disc of the hyomandibula and the "opercular process"; the rest of it dissolves. (4) The tr.hyomandibularis facialis usually runs between the laterohyal and the rest of the hyomandibula (the epihyal, cf. below); however, in actinopterygians having only a small laterohyal on top of the epihyal it runs ventrally to the laterohyal (*Polypterus* and *Acipenser*), i.e. it does not pierce the hyomandibula**. (5) Judging from the differentiation of the homologous dorsolateral blastemas in the characidean branchial arches, the laterohyal probably represents modified dorsal gill rays***. (6) The anterior articular head in holosteans and teleosteans as well as the articular disc in *Acipenser* are accordingly not a suprapharyngohyal, as HOLMGREN suggested.

Exactly *how* the dorsal hyal rays have been *transformed phylogenetically* is difficult to conclude from the ontogeny of recent material. Parallels seem, however, to appear in *Polypterus* and selachians. In adult material of *Polypterus* ALLIS (1918) has found that the gill rays of the first three branchial arches fuse at their bases "to form a practically continuous bar of cartilage." Principally the same conditions appear also in the selachian hyal arch: in rays a pseudohyoid forms by fusion of the bases of the hyal rays (cf. p. 308), and in sharks extrahyals and extrabranchials differentiate****, which represent modified hyal rays (RATHKE 1839; GEGENBAUR 1872, 1898; DOHRN 1884; HASWELL 1884; FÜRBRINGER 1903; ALLIS 1923; GOODRICH 1930; HOLMGREN & STENSIÖ 1936; EL-TOUBI 1952).

As to the extrahyals most authors are nowadays of the opinion that even these dorsal and ventral extravisceral cartilages are to be considered as transformed gill rays. EL TOUBI has shown ontogenetically in *Squalus acanthias* that the two extrahyals are composed of two portions, a hyal ray proximally and a transverse row distally. The latter is composed of pieces of cartilage, separating from the tips of other hyal rays.

These two types of transformed hyal rays, the pseudohyoid and the dorsal extrahyal, principally can be distinguished by means of their way of fusion: in the pseudohyoid there is a proximal, in the extrahyal a distal fusion of the hyal rays. However, they have in common one important thing—both are constituted by transformed hyal rays.

* In *Protopterus* and *Lepidosiren* the dorsal part of the hyal arch is reduced, cf. p. 309.

** Cf., however, *A.güldenstaedti,* p. 321.

*** It can be added that acanthodes (WATSON 1937, 1951) and lower actinopterygians, e.g. the palaeoniscid *Acrorhabdus,* had a large hyoid gill. In the latter fish the hyal gill rays extended from the opercular process and downwards (STENSIÖ 1921 Pl. 31, Fig. 4).

**** However, "a comparison with sharks will show that a pseudohyoid is foreshadowed in them" (DE BEER 1937, p. 70).

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All these examples show that secondary transformations of gill rays may occur even in recent fishes. If the interpretation of the laterohyal set forth above is correct, the selachian pseudohyoid, extrahyals and extrabranchials should principally be homologous with the laterohyal and its derivatives in teleostomes. The difference is that the pseudohyoid and the extrahyals seem to represent different functional adaptations to the special respiration type in selachians (cf. Woskoboinikoff 1932; Bertin 1958; and others). Yet they are important for this account, as they show that hyal gill rays may join and transform into structures of quite another shape.

Another example of this principle is given by Holmgren (1940, 1943) in the selachian mandibular arch. He found that the selachian processus oticus internus is formed in an analogical way as the pseudohyoid in rays (p. 338) and therefore he suggested it to be a pseudoquadrate, homologous with the pseudohyoid.

The position of the extrahyals laterally to the hyal aorta and the visceral muscles has caused Rathke (1839) and others to homologize them with the external branchial arches in *Agnatha*. This theory was contradicted by e.g. Goodrich (1909), Sewertzoff (1916—1917), de Beer (1937) and Jarvik (1954). However, this fundamental problem seems to need a special investigation, if possible even with regard to results from the experimental embryology. The theory is therefore not commented upon here.

Concerning the *homology* of the rest (the ventromedial portion) of the teleostomian hyomandibula is to point out that according to Holmgren (1943) it is an *epihyal*. If compared with the characidean branchial arches this theory seems to be acceptable. On the other hand, judging from these arches it is hard to accept his opinion that the proximal end of the epihyal, which in early embryos is present as "an inconsiderable medial process", is homologous with an *infrapharyngohyal* in *Acipenser, Amia* and *Lepisosteus*. Actually, the top of a characidean epibranchial initially always has that shape and position, i.e. it is situated below the dorsal part of the efferent visceral artery (cf. Fig. 21) and has dorsally a blastemal medial process. It is then to expect that also the epi*hyal* has the same shape and position in relation to the efferent visceral artery. Further, considering the infrapharyngobranchials they certainly have principally that position in relation to the visceral arteries. However, they form later and by means of the tips of the epal elements growing anteromediad. Finally they chondrify separately (Fig. 31). No such hyal element has been traced in either characids or in the other actinopterygians investigated here (cf. above).

It is interesting in this connection to note that immediately rostrally to the hyomandibula the *lateral commissure* is formed. However, contrary to that in *Neoceratodus* and (?) *Eusthenopteron* (p. 314) the lateral commissure in *Hepsetus* and other recent actinopterygians does not separate from the top of the hyal arch (p. 245). It may be suggested, however, that during the long lapse of time between on one hand early dipnoans and crossopterygians and on the other

recent higher teleostomians, the lateral commissure has been modified*—presumably in relation to strong adaptive requirements—so that later embryonic stages are reached by a different developmental route than that followed by ancestral groups.

The general conclusions of this investigation of the hyomandibular morphology in dipnoans and teleostomes may be summarized as follows.

Of the dipnoans *Neoceratodus*** at early stages has a hyomandibula of a primitive type, since it then seems to consist of an epal and a fused infra-suprapharyngeal element. Dorsolaterally connected with the epihyal there is, as in teleostomes, a laterohyal blastema. In the further differentiation, however, specialisations will appear: the infra-suprapharyngeal rudiment chondrifies independently and segments off, joining the neural cranium laterally to the jugular canal, i.e. in a position comparable to the lateral commissure in teleostomes. However, the hyal arch instead gets, via the laterohyal, another dorsal connection with the neural cranium dorsally to the jugular vein and the lateral commissure. The laterohyal chondrifies separately or in connection with the epihyal and posteriorly it develops a small opercular process, which often also chondrifies independently.

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The teleostomian hyomandibula initially seems to agree in principle with that of *Neoceratodus*. Immediately behind the spiracular primordium there lies along the pharynx a rudiment which, if compared with the branchial arches, seems to be an epal element. Dorsolaterally connected to this there is a subepidermal laterohyal, homologous with the dorsolateral blastemas of the characidean branchial arches producing gill rays and principally also with the modified hyal rays in sharks (dorsal extrahyal) and rays (the pseudohyoid). Contrary to *Neoceratodus,* however, an abbreviation and deviation seems to have appeared in the teleostomian ontogeny, since the infra-suprapharyngohyal here primarily develops directly from the neural cranium (the lateral commissure) just in front of and medially to the top of the hyal arch.

This theory principally lends support to the rather constructive theory of Allis (p. 316) as well as to the theory of de Beer founded on a comparison with the mandibular arch (p. 317). On the other hand it only partially agrees with the theory of Holmgren (p. 317).

Furthermore, if this interpretation is correct the *adult* hyomandibula in *Dipnoi* (*Neoceratodus*) and *Teleostomi* seems to consist of the same elements, an epal and a laterohyal one. However, as the dipnoans have autostyly like *Chimaera* (p. 309) their hyomandibula is reduced in *Protopterus* and *Lepidosiren* and is comparatively small in *Neoceratodus*. In teleostomians, on the contrary, there is another type of suspension (hyostyly) and the hyomandibula has developed more strongly.

* Probably a combination of abbreviation and deviation of the ontogeny (cf. Starck 1955; de Beer 1958; and others).

** In *Lepidosiren* and *Protopterus* the hyomandibula is reduced.

If compared with the selachian hyomandibula as described by HOLMGREN (p. 308), dipnoans (*Neoceratodus*) and holocephalians seem to have a hyomandibula with all elements persisting. However, in holocephalians the elements persist in the adult, whereas in *Neoceratodus* the fused pharyngeal elements segment off already at an early embryonic stage. The epihyal and pseudohyoid in rays seem principally to be homologous with the adult hyomandibula in *Neoceratodus* and teleostomes, whereas the sharks are said to have as well a suprapharyngohyal fused to the epal element; if so, their hyomandibula is not homologous with that of *Neoceratodus* and teleostomes (cf. Fig. 81).

When finally considering the problem of the different relations to the jugular vein—in selachians the hyomandibula articulates ventrally (medially) to the jugular vein and in teleostomians dorsally (laterally) to it—the different authors have given different answers. SCHMALHAUSEN (1923), DE BEER (1924, 1925), SÄVE-SÖDERBERG (1936) and ROMER (1937) suggested that the original hyomandibula had two articular heads, one on each side of the vein. In selachians the ventromedial head, in teleostomians the dorsolateral head was then retained, the other being reduced. Since SÄVE-SÖDERBERG and ROMER stated that this original hyomandibular type was present in crossopterygians, and (SÄVE-SÖDERBERG) even in cephalaspids, this theory seemed to be well founded.

Nevertheless objections have been made. NIELSEN (1942) and HOLMGREN (1943) objected that in *Acipenser* both articulations between the first branchial arch (its infra- and suprapharyngeal element) and the neural cranium lie ventrally to the jugular vein. No support of the theory could then, according to HOLMGREN, be found in the morphology and position of the branchial arches of recent actinopterygians.

To this is to be added, however, that in the present account it has been shown that in *Acipenser* the first suprapharyngobranchial certainly articulates ventrally (medially) to the jugular vein but also that the *second* suprapharyngobranchial articulates *dorsally* (laterally) to it (p. 305). As the two anterior infrapharyngeal elements always articulate ventrally to the vein, the two anterior branchial arches in Acipenser hence either have the dorsal articulations ventrally to the vein or on each side of it.

On palaeontologic material STENSIÖ (1925) suggested in this connection that the hyomandibula might move from the selachian position (ventrally) to the teleostome position (dorsally) with regard to the jugular vein by passing over the lateral commissure as over a bridge. This was also indirectly shown by WATSON (1925), who illustrated one single articular facet in *Osteolepis,* situated on the lateral commissure. HOLMGREN (1943) added that also in palaeoniscids the position of the hyomandibular facet is suggestive of shifting. He therefore suggested, like STENSIÖ, that "by means of the lateral commissure the two heads shifted dorsal". He also compared the crossopterygian hyomandibula with that of holosts and teleosts: "The conclusion from these statements must be that the

upper articulation of the crossopterygian hyomandibula is between a hyomandibular ray* and the neural skull, thus secondary. This ray has gained support on the neurocranium dorsally to the head-vein, the lower articulation being the primary, comparable with that in Selachians".

When discussing the problem of the different relations of the hyomandibula to the jugular vein in fishes it should be held in mind that the two hyomandibular articulations can shift (cf. above) and that in *Acipenser* the relationship of the suprapharyngobranchials to the jugular vein is not fixed (p. 305), as HOLMGREN and others have suggested. The fact that the selachian and teleostomian hyomandibula articulates differently in relation to the jugular vein should therefore not a priori be considered as a fundamental difference. A difference of their hyomandibulas should then not be explained in the way SCHMALHAUSEN, DE BEER and others have done. However, the conditions especially in *Neoceratodus* and *Hepsetus* really make it probable that the difference might be explained by assuming that their hyomandibulas are not completely homologous: the teleostomian hyomandibula dorsally articulates by means of transformed hyal gill rays (Fig. 81) (suprapharyngohyal SCHMALHAUSEN and others; suprapharyngohyal + transformed hyal ray bar HOLMGREN), not by means of e.g. epal (rays) or suprapharyngeal elements (sharks) (p. 308).

The ontogeny of the hyomandibula in *Neoceratodus* (pp. 310—315) gives indications of how the teleostomian hyomandibula may have evolved p h y l o g e n e t i c a l l y**:

(1) The hyal arch primarily consisted of separate epi-, infrapharyngo- and suprapharyngohyals. The two pharyngeal elements articulated ventrally to the jugular vein. Gill rays differentiated from the subepidermal (dorsolateral) blastema and stiffened the dorsal portion of the hyal gill. This was situated on the caudal margin of the epihyal. (Partly hypothetic).

(2) The infra- and suprapharyngohyals joined, segmented off from the rest of the arch and then fused with the neurocranium as the lateral commissure. (Early stages of *Neoceratodus* and ? *Eusthenopteron****).

(3) The hyal gill simultaneously disappeared; *the dorsal hyal rays then connected with the lateral side of the neurocranium dorsally to the jugular vein and became modified*, fusing with each other and with the epihyal. This is the laterohyal. Most of the other hyal rays disappeared. (*Neoceratodus* 12.6 mm, Fig. 72).

* According to SCHMALHAUSEN and others it should be a suprapharyngeal element.

** These different stages probably occurred partially simultaneously and without any distinct limits. In this connection, however, a division of the evolution into different stages is appropriate.

*** JARVIK suggests the lateral commissure in *Eusthenopteron* to be only the suprapharyngeal element, the infrapharyngeal being connected with its base and extending rostrally as the otic shelf. This is said to correspond to the otic shelf in selachians (absent in dipnoans and actinopterygians). According to HOLMGREN (1943), however, the selachian otic shelf belongs to the spiracular complex. The homology is then doubtful. It cannot be excluded that the infrapharyngohyal in *Eusthenopteron* corresponds to the ventral part of the lateral commissure in dipnoans and actinopterygians.

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(4) Functional adaptations to special respiration and suspension types then modified the laterohyal into different shapes with one or two articular heads or opercular processes. (*Dipnoi, Teleostomi*).

In selachians transformed hyal gill rays are also present (the extrahyals in sharks and the pseudohyoid in rays) but they do not take over the dorsal articulation of the hyomandibula. This articulation hence remained ventrally to the jugular vein.

Symplectic

The symplectic has for a long time been known to exist in actinopterygian fishes. It was found in *Acipenser* by e.g. PARKER (1882) and in *Salmo trutta* by STÖHR (1882). The latter author described the symplectic as a primarily independent element lying close to the hind end of the palatoquadrate and later fusing with the hyomandibula. This could not, however, be confirmed by HOLMGREN (1943) in *Salmo salar*. He instead found that the symplectic is initially broadly connected with the "anteroventral border of the lower part of the hyomandibula". This position it usually has in teleosts (cf. DE BEER 1937), *Amia* (PEHRSON 1922), *Lepisosteus* (HAMMARBERG 1937) and *Polypterus* (MOY-THOMAS 1933).

From the selachians a symplectic was not reported until HOLMGREN (1940, 1943) described an early separate element, connecting the mandibular arch with the hyomandibula in sharks and rays; this element "undoubtedly must be a mandibular arch ray". He then proceeded: "In older embryos this ray fuses on to the hyomandibula and disappears as a separate element. In *Potamotrygon* and *Mobula* there are two such rays present which persist in the adult stage as separate cartilages (GARMAN 1913, HOLMGREN 1942) ... the possibility thus seems not to be excluded that there are different symplectics, i.e. consisting of rays belonging to the palato-quadrate and to the mandible respectively".

This theory of a mandibular ray origin of the symplectic was earlier proposed by ALLIS (1915) in teleosts. ALLIS pointed to the observations of STÖHR and maintained that if the teleostean symplectic is not simply a ventral process of the hyomandibula "it must be looked for in other cartilages of the region, and it would seem as if it must be a specially modified ray of the mandibular arch". As a support for this idea Allis stated that the symplectic frequently fuses with the hind end of the palatoquadrate, and that in adult teleosteans the symplectic lies medially to the afferent pseudobranchial artery, which is the position a mandibular gill ray should have.

However, these arguments for a mandibular gill ray theory seem to be vague. In the teleostean branchial arches the afferent artery always runs between the two rows of gill rays (GOODRICH 1930; and others). ALLIS's last argument then can be valid even if the symplectic (gill ray) had been situated laterally to the afferent pseudobranchial artery. This argument accordingly has no value in this connection.

As to ALLIS's other arguments the following may be disputed. In *Hepsetus* the symplectic is as in *Salmo salar* (Fig. 76) formed as an anteroventral process of the hyomandibula (Figs. 1, 6), not a posterior process of the palatoquadrate as it would have been if it is a mandibular gill ray. Judging from the branchial arches this seems to be of primary importance and is not influenced by the fact that at the 8 mm A stage it chondrifies separately (Fig. 12). In other teleosts such as *Gasterosteus* (SWINNERTON 1902) and *Anguilla* (NORMAN 1926) as well as in holosts (PEHRSON 1922, HAMMARBERG 1937, HOLMGREN 1943) (Fig. 77) the symplectic is also initially continuous with the hyomandibula. The same is also true for *Polypterus* (ALLIS 1922) (Figs. 79, 80) and *Acipenser* (DE BEER 1925, SEWERTZOFF 1928) (Fig. 78). It then chondrifies independently or in connection with the hyomandibula; it is, however, still fused with the hyomandibula but in *Acipenser,* where it segments off and, as in certain palaeoniscids (NIELSEN 1942), articulates with the palatoquadrate and with the lower jaw. In adult holosts and teleosts it is often separately ossified and secondarily connected with the quadrate bone in front. In rhipidistian crossopterygians an ossified symplectic is probably not present (JARVIK 1954), whereas in coelacanthids a strong and partially ossified symplectic connects with the mandibular arch (MILLOT & ANTHONY 1958).

This survey has shown that the teleostomian symplectic is primarily an anteroventral process of the hyomandibula (Fig. 81). Secondarily, however, it may segment off and articulate or connect with the mandibular arch. This is probably an adaptation related to the teleostomian respiration type cf. WOSKOBOINIKOFF 1932) and not an indication of an earlier existence as a mandibular ray. The one or two mandibular rays in selachians that secondarily may fuse with the hyomandibula should then not be homologous with the teleostomian symplectic.

Stylohyal

The presence of a stylohyal (interhyal) is also characteristic of the actinopterygians. Concerning its homology there are many theories. ALLIS (1922) for example suggested it to be an epihyal, whereas STÖHR (1882) and SEWERTZOFF (1928) considered it to be a segmentation from the upper end of the ceratohyal. ALLIS's theory does not agree with the information brought forth in this investigation, according to which instead the symplectic and ventral part of the hyomandibula represent the epihyal (cf. above and Fig. 81).

HOLMGREN (1943) presented another explanation, founded on a comparative embryologic investigation of *Acipenser, Lepisosteus, Amia* and *Salmo.* In these species he found that "the definitive stylohyal consists of two parts, a medial and a lateral. The medial portion is part of the primary hyoid arch, the lateral during development is added to the former and may be explained as a branchial ray belonging to the epihyal portion of the hyomandibula. The relative primary indepence of the lateral part (ray element) of the stylohyal seems after chondrifi-

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cation to cause the segmenting off of the medial (also chondrifying) portion from the epihyal and the ceratohyal respectively".

The present investigation has given indications that HOLMGREN's *hyal ray theory* may be correct. It was shown in chapter III that in *Hepsetus* 6.5—7.3 mm the stylohyal is beginning to differentiate from two parts, a medial and a lateral (cf. Fig. 9). The former is thicker and represents the middle part of the continuous inner skeletal arch. Laterally this part is connected with the subepidermal blastema. As this dorsally continues with the more densely nucleified laterohyal, the lateral part of the stylohyal accordingly may represent a gill ray blastema (cf. p. 307). These early stages of *Hepsetus* agree with the 20 days 5 hours stage of *Salmo salar,* described by HOLMGREN.

At the 8 mm stages of *Hepsetus* the lateral part of the stylohyal chondrifies together with the medial part into one piece. There are no traces of a direct cartilaginous connection between the hyomandibula and the ceratohyal anteriorly to the stylohyal, as was described by HOLMGREN in *Salmo* 26 days 13.5 hours. Further, it is hard to discern if the stylohyal belongs to the epihyalosymplectic or to the ceratohyal or both.

The hyal ray theory has also supports in dipnoans. In the early ontogeny of *Neoceratodus* a thick blastemal knob is formed on top of the ceratohyal (Fig. 71). According to its position and shape it seems to be a stylohyal rudiment. As in *Hepsetus* this knob consists of cells from both the skeletal arch and from the thin subepidermal blastema. As this also here is dorsally continuous with the more densely nucleified laterohyal (p. 315), the lateral part of the symplectic rudiment in *Neoceratodus* for the same reasons as in *Hepsetus* may represent a modified hyal ray.

Mandibular arch

When studying the literature about the homology of the mandibular arch, it is striking how different authors have come to such different results concerning selachians and teleostomians. In the former ones the mandibular arch has been considered to be rather uncomplicated, whereas, in teleostomians there might be included both one and two premandibular arches as well. The reason for this seems to lie in the different shape of the palatoquadrate. As this element is rather short and club-like in selachians (except for the notidanids) it has been difficult to imagine it to include even premandibular elements. In teleostomes, on the other hand, the palatoquadrate has a long rostral process, the processus pterygoideus, which has made such an idea more tempting.

Most attempts to homologize the mandibular arch in fishes has been made in *selachians.* The literature on this subject is summarized and discussed by SEWERTZOFF (1923) and HOLMGREN (1943). Generally there are three opinions, viz. that it consists of (1) only an epal and a ceratal element (GEGENBAUR 1872), (2) a pharyngeal, an epal, a ceratal and a hypal element (SCHAUINSLAND 1903; WOS-

KOBOINIKOFF 1914; SEWERTZOFF 1923; JAEKEL 1926; and others) and (3) a suprapharyngeal, an infrapharyngeal, an epal, a ceratal and a hypal element (HOLMGREN 1943).

As to the homology of the *hypomandibula* there are also different ideas (cf. the cited literature above). DE BEER referred to a cartilage nodule found in the symphysis in *Hexanchus* (FÜRBRINGER 1903; SEWERTZOFF 1923), *Chlamydoselachus* (FÜRBRINGER 1903) and *Somniosus* (*Laemargus*) (WHITE 1896). Such a symphysial cartilage has on the other hand not been found either by ALLIS (1923) or GEGENBAUR (1872).

HOLMGREN's thorough investigations of selachians (1940, 1943) showed that a symphysial tissue is always present in the embryos and that this tissue is apt to chondrify (in rays it instead forms a "tendinous mass"). As in the symphysis of *Etmopterus* and *Squalus* there may be two different elements present, viz. an unpaired medial and a paired lateral. Contrary to DE BEER he considered the medial one to be a *basimandibula,* whereas the hypomandibula instead was said to be represented by the lateral element and the *ceratomandibula* by the rest of the meckelian cartilage (Fig. 82 C).

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As a support for this theory HOLMGREN also referred to VAN WIJHE's statement (1922) that the lower jaw in *Squalus acanthias, Pristiurus, Scyllium* and *Torpedo* chondrifies from two centres, and that in *Acanthodes Bronni* (REIS 1896, JAEKEL 1926) and *Leiosteus* (STENSIÖ 1934) it has two separate ossifications. The latter evidence should, however, be treated with caution, because in many teleostomes the ceratohyal is also ossified into two bones (NIELSEN 1942; DEVILLERS 1958; and others). As is well known neither of these bones, however, is the hypal element. It seems hence not probable that the two ossification centers in the mandible also signify two earlier chondrification centers (cf. p. 336). As to the symphysial cartilage, finally, it must be kept in mind that there can be formed a variable number of cartilages in the symphysis. This fact inclined FÜRBRINGER (1903) to regard them as vestigial mandibular rays. Such gill rays were developed in acanthodians (JAEKEL 1925). Further, it has to be remembered that a symphysial cartilage seems to be very sporadically present in adult sharks, and in rays it is substituted by a tendinous mass (cf. above). These facts may indicate that the symphysial cartilage(s) might be accessory (cf. SCHAFFER 1930). It therefore seems necessary to make a comparative histological analysis of the selachian symphysial cartilage as well as of the basihyal and the basibranchials before a definitive statement about the homology can be made.

No hypal or basimandibular elements seem to be described in *teleostomes.* The present investigation has shown, however, that in *Hepsetus* a symphysial blastema is formed at early stages (Figs. 12, 14, 15). As in rays it does not chondrify but instead forms a tendinous tissue*. Whether this represents a transformed basi-

* The symphysial blastema has nothing to do with the later chondrifying prelingual cartilage (Figs. 33, 39, pl.c.). This consists of fibrous cartilage and is probably accessory.

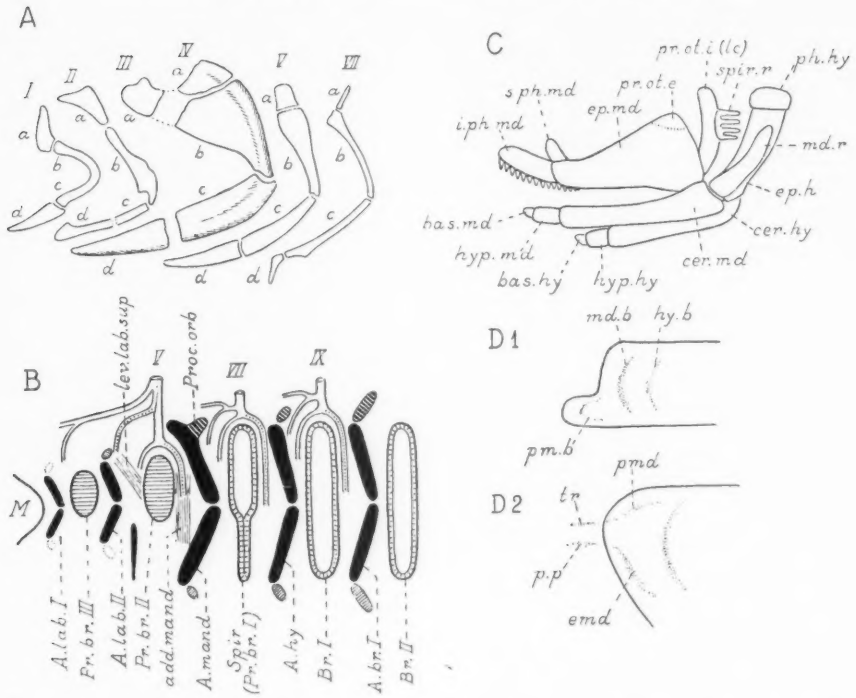

Fig. 82. Five diagrams illustrating the opinions of (A) JAEKEL, (B) SEWERTZOFF, (C) HOLMGREN and (D1, D2) ALLIS concerning the prootic visceral arches in gnathostomes. From JARVIK (1954, Fig. 38). For indications and letterings see the text and JARVIK.

mandibula is hard to say. In this case an experimental investigation should be made to ascertain if the basihyal and basibranchials exposed to variable changes in their environments might be secondarily transformed into tendinous tissue.

The problem of the *upper jaw* and its homology has been of great interest during the last decades. Especially the question of what the *pharyngomandibula* is, has been subject of numerous speculations and researches. One hypothesis has already been discussed in connection with the trabecular problem, viz. ALLIS's (1923) suggestion that the polar cartilage is the pharyngomandibula (Fig. 82 D, pmd) and (1931) that the trabecle (tr.) and the palatine process of the palatoquadrate (pp.) in selachians are the dorsal and ventral halves, respectively, of the premandibular arch. This suggestion, however, is partially purely theoretical (cf. p. 289), partially founded on the brief description of HALLER (1924). The latter mentions a dorsal "Kieferaugenspaltstück" in selachian embryos, which he homologizes with a pharyngomandibula. It is said to disappear in adults. Judging from HALLER's description of its relationship to nerves and vessels, the ele-

ment seems to be the processus oticus internus according to HOLMGREN (1943) and therefore "it could not be a pharyngomandibular at all" (cf. below).

SEWERTZOFF (1923) (Fig. 82 B) on the other hand suggested the pharyngomandibula in sharks (proc.orb.) to be represented by the orbital process. During the following year he changed his mind, however, and considered the small cartilage, which had been found by IVANZOFF (1877) in the "ethmoid ligament" of *Scaphirhyncus* and by DISLER (SEWERTZOFF & DISLER 1924) at the upper end of the orbital process in *Somniosus, Mustelus, Scyllium* and *Squalus,* to be the pharyngomandibular element.

HOLMGREN (1943) also investigated these species but concluded that the cartilage nodule in *Somniosus* and *Squalus* (if ever present) must be accessory and, further, that the cartilage found in *Mustelus* and *Scyllium* does not join any orbital process. Instead it constitutes itself that process. The two former cartilages are obviously developed in the orbital ligament, and therefore their homology with a pharyngeal element seems to be rather questionable.

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JAEKEL (1926) (Fig. 82 A) found that the palatoquadrate in *Acanthodes bronni* ossifies into separate bones, an anterior autopalatine, a posterior quadrate and a dorsal metapterygoid between them*. He therefore suggested that they represent different visceral elements: a "pharyngomaxillar" (Fig. 82 A, IIa) (pharyngopremandibula), an epimandibula (IVb) and a pharyngomandibula (IVa), respectively. An important objection against this hypothesis is that in osteolepidean crossopterygians and in palaeoniscids the palatoquadrate usually had still more bones. In e.g. *Megalichthys* (WATSON 1919) and *Saurichthys* (STENSIÖ 1925) a continuous *series* of endochondral suprapterygoid bones extended along the whole dorsal margin of the upper jaw. By investigating a large material of *Glaucolepis* NIELSEN (1942) found "individuals with the palatoquadrate entirely ossified into one piece, others with two or three smaller ossifications ... and others again with entirely unossified palatoquadrates". NIELSEN proceeded: "Quite probable the degree of ossification ... depends on the age of the fishes and it serves no purpose whatever to insert in the generic diagnosis ... whether the palatoquadrate contains several small or one large ossification" (cf. p. 334).

HOLMGREN (1943) (Fig. 82 C) brought a new element into the discussion, when dealing with the problem from the assumption that the mandibular arch in fishes is originally divided in the same way as the teleostomian branchial arches, i.e. with two pharyngeal elements dorsally instead of one. The *suprapharyngomandibula* (s.ph.md.) in selachians should then be represented by the orbital process (cf. SEWERTZOFF above), whereas the independently formed pars palatina is the *infrapharyngomandibula.*

None of these theories was founded on the teleostomian palatoquadrate.

* As is well known these bones are also formed in coelacanthids (STENSIÖ 1921, MILLOT & ANTHONY 1958) and actinopterygians (GOODRICH 1930 and others).

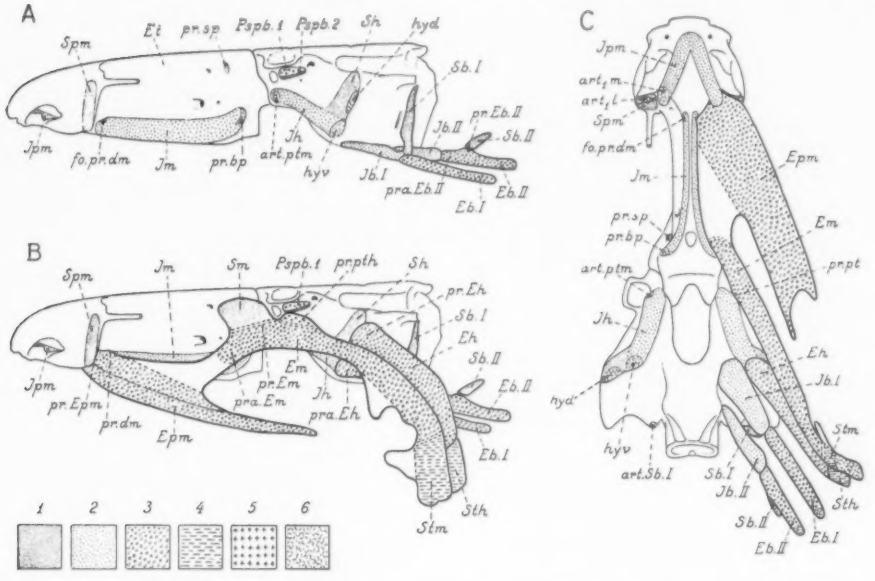

Fig. 83. *Eusthenopteron foordi.* The approximate position of the suprapharyngeal, infrapharyngeal and epal elements of the prootic arches. A, neural endocranium and the dorsal parts of the first and second branchial arches, lateral view; B, C, the same parts of the endoskeleton as in A, with addition of the palatoquadrate bone, the hyomandibula and the stylohyal. B, lateral, C, ventral view. From JARVIK (1954, Fig. 39). For indications and letterings see the text and JARVIK.

HOLMGREN had, however, earlier in his paper (1943) suggested that "the palato-quadrate of the teleosts is built like that of the sharks, consisting of 1) a palato-quadrate proper*, answering to the ontogenetically primary palato-quadrate in sharks, 2) an articulation ligament corresponding to the orbital ligament of sharks**, 3) a symphysial (palatine) portion, fusing on to the lower surface of the ethmoid plate and corresponding to the palatine part of the palato-quadrate in sharks".

Recently JARVIK (1954) has agreed with HOLMGREN's theory of two pharyngeal elements being present also in the prootic arches in teleostomes. On basis of studies of *Eusthenopteron* he came to the conclusion that the dorsal part of the mandibular arch is not situated in the ethmoidal but in the orbito-temporal region (Fig. 83), the processus ascendens being the suprapharyngomandibula (Sm) and the suborbital ledge + the basipterygoid process (the ectomesenchymal part of the trabecle, cf. however pp. 293—303 representing the infrapharyngomandibula (Im). This element has hence secondarily been incorporated in the neural cranium (cf. p. 303).

* The pars quadrata + processus pterygoideus.
** And hence the ethmo-palatine articulation = the orbital articulation in sharks (p. 247).

As regards the cyclostomes traces of an internal mandibular arch have been described both in *Myxine* (HOLMGREN 1946) and *Petromyzon* (JOHNELS 1948).

From this survey of the literature it is evident that the problem of the composition of the palatoquadrate and adjacent structures in teleostomes is far from solved.

In chapter III it has been shown that the primordial mandibular arch in *Hepsetus* agrees principally with the other visceral arches (Fig. 4). It consists of a continuous blastemal arch medially which, as in the other visceral arches, is dorsolaterally connected with a subepidermal blastema of another histological type (p. 306). At the 7.3 mm stage this dorsolateral blastema has come in contact with the base of the postorbital process of the neural cranium. As it also has the same relationship to the m.constrictor dorsalis and the m.adductor mandibulae as the primordium of *processus oticus externus* has in notidanid selachians (cf. HOLMGREN 1943 and p. 214), it seems to be homologous with this process. It is interesting to note that HOLMGREN suggested that this process may be "looked upon as a portion of the palato-quadrate ray bar of which the posterior portion gives rise to the spiracular complexity". If the homology with the dorsolateral blastema of the mandibular arch in *Hepsetus* is correct, HOLMGREN's theory has got supports also in actinopterygians (*Hepsetus*).

In *Hepsetus* the processus oticus externus (dorsolateral blastema) chondrifies only at its connection with the pars quadrata (Figs. 12, 15, pr.ot.e.). The rest of it dissolves. The chondrified base is still seen at the 8 mm B stage, but it is rather diminutive and is later completely embodied by the pars quadrata and cannot be traced any more.

Instead of the processus oticus externus there is, however, at the 8 mm B stage another palatoquadrate process forming that will remain until adult stage. This process is usually called processus oticus or processus metapterygoideus in actinopterygians. As it is situated medially to the m.constrictor dorsalis (m.levator arcus palatini) it should represent the *processus oticus internus* in selachians (Fig. 15, pr.ot.i.) (HOLMGREN 1943). In *Etmopterus* and *Heptanchus* it occurs even simultaneously with the external otic process (HOLMGREN 1943). It is caudally connected with the prespiracular (palatoquadrate) rays (Fig. 82 C, spir. r.), developed from a common mesenchyme situated in connection with the palatoquadrate. "On the lateral or posterior border of the processus oticus (internus) portion the spiracular rays are placed as in the hyoid arch in rays, where the hyoid rays are attached to the pseudohyoid, which has originated through fusion of the basis of the hyoid rays. The processus oticus (internus) might thus be considered as a 'pseudo-palatoquadrate'" (HOLMGREN 1943) (cf. p. 327).

It is not possible to find any contributions to this theory in the characidean material, nor to HOLMGREN's theory that the internal otic process is homologous with the lateral commissure in teleostomes. Actually, it is situated anterior to the spiraculum, whereas the lateral commissure forms posterior to it. Further, a rein-

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vestigation of his material shows that the membrane (lamella), that in *Amia* and *Salmo* was said to join the palatoquadrate and the trabecle, caudally only connects with the lateral commissure and does not seem to "arise through the chondrification of the lamella" (HOLMGREN 1943, pp. 37, 42). As in *Hepsetus* the membrane certainly is connected with the lateral commissure, but the latter chondrifies from the neural cranium—not from the membrane (p. 245).

HOLMGREN's idea of a visceral origin of the lateral commissure was not new. It had earlier been claimed by ALLIS (1914 a, b, c, 1918, 1923). ALLIS's evidences of this theory were, however, founded on imperfect information on *Neoceratodus;* they are discussed on p. 314. Objections had been made earlier also by DE BEER (1926), who considered the lateral commissure to be an element formed from three different processes of the neural cranium. This theory is discussed on p. 244. The present investigation has shown that the primordial lateral commissure of higher actinopterygians usually arise only from one process, the prootic (Fig. 8, pr.p.ot.). This grows mediad and joins the anterior end of the basiotic lamina. The chondrification of the commissure then sets in peripherally. If the blastemal part of it is not taken into consideration, the commissure now consists of a prootic (dorsally) and a postpalatine process (ventrally) as DE BEER maintained. The third process, the basitrabecular, is not present in *Hepsetus.*

The morphological relationship of the processus oticus internus in selachians thus justifies a homologization not with the lateral commissure but with the inner otic process in *Hepsetus.* The lateral commissure is probably a hyal formation instead (p. 328).

Anteromedially to the processus oticus internus there is a process in *Hepsetus* that constitutes the dorsomedial end of the pars quadrata and that at early stages is connected with the polar cartilage. It should hence be the *processus basalis* in other fishes (Figs. 4, 6, 12, 13, 14, 25, 33, pr. bas.), e.g. acanthodians (WATSON 1938), palaeoniscids (STENSIÖ 1925; WATSON 1925, 1928; NIELSEN 1949) and embryonic stages of *Lepisosteus* (HAMMARBERG 1937) and certain selachians (HOLMGREN 1940, 1943). The rr.maxillaris and mandibularis trigemini divide at the hind margin of the process (Fig. 18). At older stages it withdraws from the polar cartilage and chondrifies; the relation to the nerves is, however, still the same (Fig. 36). If compared with the ontogeny, morphology and relation to the visceral nerves and aortas of the branchial arches, the pars quadrata seems to represent an *epimandibula* (Fig. 85, e.md.), ending frontally in the basal process.

Like the ventral portion of the epihyal (the symplectic) the ventral part of the epimandibula points rostroventrad. Further it is at early stages continuous with the coronoid process (stylomandibula cf. below) of the meckelian cartilage (Fig. 14). This ontogenetical type resembles that of the symplectic, above all in *Lepisosteus* 9.3 mm (Fig. 77) and *Acipenser güldenstaedti* 9 mm (Fig. 78). For comparative anatomical reasons the ventral part of the epimandibula may correspond

to a symplectic and should therefore be called a *mandibulo*-symplectic (Fig. 85, md.sy.). In support of this theory may also be mentioned that the corresponding portion is a separate bone in coelacanthids (JARVIK 1954). Further, it should be noted that the hyosymplectic, as is well known, usually also ossifies independently.

If these interpretations are true it should for comparative anatomical and ontogenetical reasons be useful to consider the large coronoid process to be a *stylomandibula* (Fig. 92, st.md.) and the meckelian cartilage a *ceratomandibula* (c.md.). No hypal element is traced in the teleostomes investigated. The basal element is dealt with on pp. 334, 335.

Comparing with *Eusthenopteron* (JARVIK 1954) and *Neoceratodus* (p. 302) the *infraphroyngomandibula* in actinopterygians may be represented by the trabecle. However, the epimandibula in actinopterygians primarily does not join the trabecle but the polar cartilage*. If the trabecle really is the infrapharyngomandibula it must hence have lost the original connection with the epimandibula (its basal process) when it became incorporated in the neural cranium. Thus, the trabecle (infrapharyngomandibula) in this respect reminds of the lateral commissure, which in *Hepsetus* has lost the connection with the hyomandibula (epi- + laterohyal). In both cases the cause might have been a combined abbreviation and deviation in the ontogeny (cf. p. 328).

Supposing that the processus oticus internus in *Hepsetus* is homologous with that of the selachians and with the otic process in other actinopterygians, and supposing that the theory of the gill ray origin is correct, there seem to be no traces of the other pharyngeal element, the *suprapharyngomandibula,* either in actinopterygians or in selachians**. SEWERTZOFF's and HOLMGREN's ideas that the selachian processus orbitalis represents a suprapharyngomandibula (p. 336) is rather improbable, as HOLMGREN (1943) has stated that their orbital articulation corresponds to the ethmo-palatine articulation in teleostomes (p. 247), and since there are reasons to believe that this articulation is situated between the epi- and suprapharyngopremandibular elements (Fig. 85, cf. below).

In *Eusthenopteron* (JARVIK 1954) there is a process, the ascending process, which has been homologized with a suprapharyngomandibula (Fig. 83, Sm). In *Neoceratodus* (Fig. 72, pr.asc.) there is also an ascending process (HUXLEY 1876; SEWERTZOFF 1902; and others) with the same relation to nerves, vessels and the basal process as in *Eusthenopteron*. For that reason the ascending process in *Dipnoi* might be a suprapharyngomandibula.

* Sometimes this connection is a real articulation between the basal process and the basipterygoid (basitrabecular, basipolar) process of the neurocranium (DE BEER 1937 and others).

** There is also a "posterior process" in *Hepsetus,* connecting with the symplectic (Figs. 14, 33, pr.p.). It seems, however, to be secondarily formed to give place to the afferent pseudobranchial artery.

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Premandibular arch

An important contribution to the question if a premandibular arch can be traced in fishes is given by JARVIK (1954). After having interpreted only the posterior part of the palatoquadrate (pars quadrata) as belonging to the mandibular arch he proceeded: "If these interpretations of the mandibular arch just given are correct, the pars autopalatina, that is, the large antero-lateral portion of the palatoquadrate (proc. pterygoideus), cannot possibly belong to that arch. This portion lies antero-laterally to the prespiracular groove (gr.psp., Figs. 31, 35)* and thus antero-laterally also to the diverticulum in the roof of the pharynx which is intepreted as a vestige of the premandibular gill-slit (p. 59), and for this reason it may be assumed that the pars autopalatina—in accordance with the views held by JAEKEL**. . .—belongs to the premandibular arch". He then gave reasons for the theory that the pars autopalatina (pterygoid process) is the epal element (Fig. 83 Epm.), the lamina orbitonasalis the suprapharyngeal element (Spm) and the ventral portion of the ethmoidal region, supporting the vomer (pars palatina of selachians, one half of the palatoquadrate commissure in actinopterygians, cf. p. 345) is the infrapharyngeal element (Ipm) of the premandibular arch.

A premandibular arch had, however, earlier been suggested also by other authors. ALLIS (1923) and JAEKEL (1926) are already mentioned (pp. 335, 336). As is shown in Fig. 82 A, JAEKEL assumed that there were originally even three premandibular arches present (I—III), the two anterior ones represented by labial cartillages.

Labial cartilages had also earlier been interpreted in this way. SEWERTZOFF (1923) (Fig. 82 B) proposed that the anterior and posterior labial cartilages each might be a pair of vestigial premandibular arches. In 1928 SEWERTZOFF extended the hypothesis also to the teleostomes, when he found that the mandibular muscle rudiment in *Acipenser* is situated medially to the maxillary bone. He then proposed this bone to represent a vestigial premandibular arch, homologous with the posterior pair of labial cartilages in selachians***.

HOLMGREN (1943, pp. 32, 33) because of certain additional reasons joined SEWERTZOFF's hypothesis.

Against the hypothesis of the labial cartilages representing old visceral bars is to be objected that neither of the authors has discussed the possibility that the labial cartilages are only accessory structures. Here a broad histological and experimental investigation is needed before the hypothesis can be accepted.

* A prespiracular groove is also described in *Porolepiformes* (GROSS 1936; JARVIK 1942, 1954) and a vestige of it in selachian (VAN BEMMELEN 1886, SEWERTZOFF 1923) and dipnoan embryos (GREIL 1912).

** Cf. above p. 336, Fig. 82 A.

*** Actually these are, according to HOLMGREN (1940, 1942), formed in front of the "anterior" ones, later shifting during the ontogeny. SEWERTZOFF accordingly should have compared the maxillar bone with the anterior labial cartilages instead.

An important evidence of the presence of an original premandibular arch is given by STENSIÖ (1927, 1932, 1942, 1958) in his investigations of the cephalaspids, pteraspids and arthrodires. He also homologized this arch with the lateral mouth plate in the larva of *Petromyzon*. Further reasons for such an opinion have been advanced by JOHNELS (1948). HOLMGREN (1946) suggested that it is possible to interpret the anterior lateral plate in *Petromyzon* and the labial cartilage in *Myxine*, respectively, as internal premandibular arches.

If the interpretations of the mandibular arch in *Hepsetus* given above is correct, the remaining portion of the palatoquadrate, i.e. the processus pterygoideus, cannot possibly belong here. This deduction is also supported by the ontogeny of the process, because at the blastemal stage it has a certain independence in relation to the pars quadrata (Figs. 7, 14, pr.pt., cf. p. 228): the nuclei are scattered and therefore easy to distinguish where the process caudally connects with the medial margin of the pars quadrata. In pars quadrata the nuclei are instead regularly arranged*. Supposing that the pars quadrata and the trabecle in *Hepsetus* really are the epi- and infrapharyngomandibula, respectively, and if it is kept in mind that lower fishes seem to have had a vestigial premandibular arch (cf. above), it seems reasonable to assume that the relatively independently forming pterygoid process is homologous with an *epipremandibula*.**

When thereupon searching for the infrapharyngeal element of the premandibular arch it is very tempting to remind of the blastemal *intermediating body*, described by HOLMGREN (1943) in *Acipenser*, *Amia* and *Salmo* and also present in *Hepsetus* (Figs. 13, 16, 23, int.b., cf. pp. 239, 248). It constitutes the anterior tip of the pterygoid process. In *Hepsetus* as in *Amia* it chondrifies separately (9 mm, 13 mm) but is still continuous with the pterygoid process. The rostropalatine articulation then arises at its medial surface (Fig. 33, rp.a.). In *Acipenser* it changes into ligament.

In such characids where the maxillary is only connected with the intermediating body, it detaches when chondrifying (p. 255, Fig. 84). In this way one or two submaxillary cartilages (SAGEMEHL 1891) are formed. As these cartilages appear in herbivorous species, SAGEMEHL suggested that this arrangement is formed to allow a considerable sideward mobility of the upper jaws and of the entire ethmoidal region***. The submaxillary cartilage(s) should hence be con-

* As the pterygoid process initially has its principal connection with the base of the epimandibula, it cannot represent the infrapharyngomandibula, if compared with the branchial arches.

** The independent chondrification of the pterygoid process in *Clupea* (WELLS 1922) and in most silurids (RYDER 1887; KINDRED 1919; SRINIVASACHAR 1953, 1956, 1957) is probably secondary (NORMAN 1926, DE BEER 1937). This is supported by SRINIVASACHAR's observations in siluroids of a pterygoid process continuous with the pars quadrata (*Arius*) and a gradual reduction to only a short process, dorsally connected with the lamina orbitonasalis (*Silonia, Pangasius, Ailia*).

*** The tendency towards formation of separate cartilages at the margin of the ethmoidal plate exists according to SAGEMEHL also in *Perca* (one), *Cobitidinae* (two) and *Catostominae* (three cartilages).

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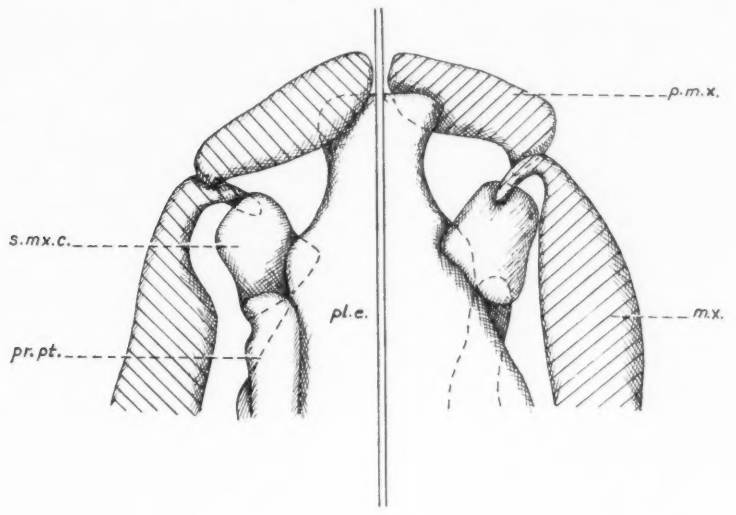

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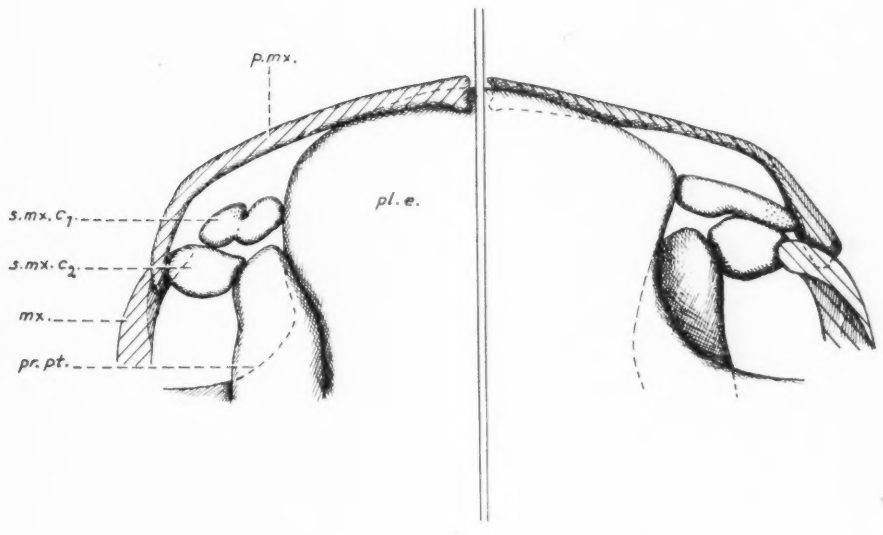

B

Fig. 84. Reconstruction of ethmoidal region of two characids in which the maxillary connects only with the submaxillary cartilage(s), not with the ethmoidal plate. Left, ventral view; right, dorsal view. 75 ×. A, *Nannocharax ansorgei,* 15 mm; B, *Citharinus citharinus* 12 mm.

sidered as an adaptation for increased mobility of the upper jaw. Thus also the rudiment of it, the intermediating body, is a secondary element.

However, HOLMGREN has also described how in *Amia* a process develops mediad from the intermediating body and below the anterior part of the trabecle; this process he considered to represent, "partly at least, a vomeral blastema". In *Salmo* it continues mediad and fuses with that of the other side, forming a *palatoquadrate commissure*. Simultaneously it fuses with the lower surface of the ethmoidal plate and becomes incorporated in the neural cranium. This commissure is also described in *Corydoras* and *Cyclopterus:* in the latter it is in part separately chondrified (HOLMGREN 1943, Fig. 33). As the pars palatina in sharks has a similar position and also sometimes chondrifies separately HOLMGREN suggested pars palatina and half the palatoquadrate commissure to be homologous and represent the infrapharyngomandibula (Fig. 82 C). The pars palatina in sharks is not incorporated in the ethmoidal region as is the palatoquadrate commissure. In the placoderms, however, STENSIÖ has suggested it to be represented by a distinct ridge on the lower side of the ethmoidal plate (region) (cf. *Cyclopterus* above). As to the crossopterygians, finally, JARVIK maintained that although no ridge or any other visible traces of the palatoquadrate commissure* are present in *Eusthenopteron* other reasons make it probable that it was developed at embryonic stages and that it represents the *infrapharyngopremandibulae*.

This conclusion is supported by embryologic evidences provided by HOLMGREN. It should be stressed that the ontogeny is of importance here: like a normal infrapharyngeal element the palatoquadrate commissure in *Amia* and *Salmo* is said to be formed by growth rostromediad from the anterior part (intermediating body) of the epal element (pterygoid process). In *Hepsetus* the palatoquadrate commissure (Fig. 17, comp.pq.) is also formed in ventrolateral connection with the intermediating body (int.b.), but it is doubtful whether it is formed in the same way as in *Amia* and *Salmo*. Anyhow the palatoquadrate commissure is similarly placed and differentiated. From its dorsal portion chondrocytes are added to the ethmoidal plate, and ventrally osteocytes form the rudiments of the vomer, the dermopalatine and the premaxillary (Fig. 17 B).

According to JARVIK the presence of these bones is also of importance for the homology: "That portions of the endoskeleton which carry the vomer, the dermopalatine and the ectopterygoid, really represent the infrapharyngeal and epal elements of the premandibular arch, is further supported by the fact that this row of tooth-bearing dermal bones is opposed to the correspondingly developed row of tooth-bearing dermal bones (the coronoids) on the dorsal side of the meckelian bone, which latter no doubt is the ceratal (and ? hypal) element of the

* Instead of the "palatoquadrate commissure" JARVIK used the term "intermediating body". This element, however, constitutes only the lateral part of the palatoquadrate commissure according to HOLMGREN (cf. *Hepsetus* Fig. 19).

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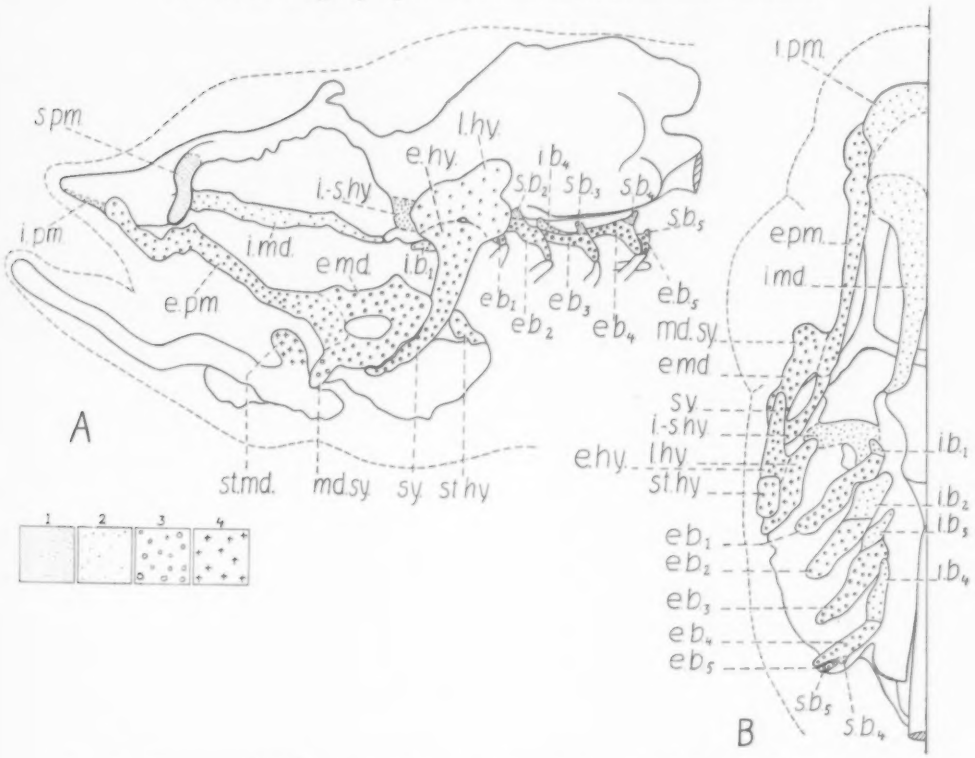

Fig. 85. *Hepsetus odoë*. The approximate position of the suprapharyngeal, infrapharyngeal and epal elements of the three prootic arches. A, chondrocranium, lateral view; B, left part of chondrocranium, ventral view. 1, suprapharyngeal elements; 2, infrapharyngeal elements; 3, epal elements; 4, gill ray portions.

mandibular arch. The fact that the dorsal elements of the premandibular arch and their teeth are situated opposite to the ventral elements and teeth of the mandibular arch is in full agreement with the conditions in the other branchial arches." The fact that a part of the palatoquadrate commissure in *Hepsetus* forms the rudiments of the vomer, dermopalatine and premaxillary thus might be of interest when searching for the infrapharyngeal and epal elements of the premandibular arch in recent fishes.

The present embryologic investigations and the comparisons made with *Acipenser, Amia* and *Salmo* as well as with *Eusthenopteron* and the arthrodires indicate that the palatoquadrate commissure in actinopterygians is homologous with the fused infrapharyngopremandibulae (Fig. 85, i.pm.).

When finally searching for the possible traces of the *suprapharyngopremandibula* it can be noted that in *Hepsetus* the epipremandibula (pterygoid process)

is primarily intimately fused also with another blastema, viz. that of the lamina orbitonasalis (Fig. 15, l.o.n.). In *Hepsetus* this element initially raises from the epal element but is free from or only loosely connected with the cartilago supraentethmoidalis (c.se.) and with the ethmoidal plate (pl.e.) (cf. p. 234). The connection with the epal element then breaks up. Dorsally the orbital articular ligament and ventrally a cartilage process is formed. This probably corresponds to the processus orbitalis in selachians (p. 247). The ethmo-palatine articulation (Fig. 33, ep.a.) is constituted by this process and the lamina orbitonasalis (cf. e.g. the siluroids, SRINIVASACHAR 1957). This articulation is homologous with the orbital articulation in selachians, according to HOLMGREN (1943).

The shape, articulation and ontogeny of the lamina orbitonasalis in *Hepsetus* thus indicate that it may represent a suprapharyngopremandibula (Fig. 85, s. pmd.). This is in agreement with the theory of JARVIK that the posterolateral part of the nasal capsule in *Eusthenopteron* is that kind of an element (Fig. 83, Spm).

Embryologic supports of this theory can also be found in other fishes. First of all HOLMGREN (1940) has shown the selachian lamina orbitonasalis to be of ectomesenchymal origin, i.e. of the same origin as the visceral skeletal arches. Further, he has suggested (1943) that it is homologous in teleostomes and sharks and develops from a rudiment, which is independent of the neural cranium (cf. *Hepsetus* above). This rudiment later chondrifies in connection with the ethmoidal plate. An independent chondrification of the lamina has, however, been described in the syngnathid group, *Cyclopterus* (UHLMANN 1921), *Siphonostoma, Syngnathus* (NORMAN 1926) and *Hippocampus* (KADAM 1958).

Considering the innervation and blood support of a premandibular arch only that fact is pointed out for the moment that theories of the innervation has been proposed by SEWERTZOFF (1923, Fig. 82 B and JARVIK and that both ALLIS (1912) and HOLMGREN (1943) have maintained that selachians as well as teleostomians possess vestigies of a premandibular aorta. There seem to be reasons for such a theory also concerning *Hepsetus*. This will, however, be treated elsewhere.

Summary and conclusions

1. The ontogeny of the chondrocranium of 10 characid species has been investigated. The *description* is based mainly on a large embryologic material of the African characid *Hepsetus odoë*, consisting of 70 stages from egg and up to 45 mm length. In addition certain new data have been added concerning the structure of the chondral skull in *Salmo salar, Amia calva, Lepisosteus osseus, Polypterus bichir, Acipenseridae* and *Dipnoi*. As a rule structural features of importance for the interpretation of the nature and composition of the chondrocranial base and the visceral skeleton have been considered in detail (p. 204).

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2. The *intention* of this work has been to follow the development of the chondroskeleton as far back as possible, because studies of the youngest stages (the blastemal and prochondral stages) are particularly important from phylogenetic point of view (HOLMGREN 1943; DE BEER 1937; and others). The relationships between the cranium and vessels, nerves and muscles have also been considered (p. 207).

3. Four stages in the *histogenesis* of the chondral elements are accounted for: the blastemal, prochondral, protochondral and chondral stages (SCHAFFER 1930). The blastemal stage is characterized by a syncytium of cells with rounded nuclei; at the late blastemal stage the osteo- and chondroblastemal nuclei stain differently in Azan (p. 207).

It is possible to make a *demarcation* of the blastemas if they are densely nucleified or surrounded by another type of cells. Only such blastemas are generally accounted for in the reconstructions (p. 208).

4. The term endomesenchyme is used here for mesenchymal cells detaching from the endomesoderm (p. 208, note 1). This is in analogy with the term ectomesenchyme, which was introduced by DE BEER (1947) and more thoroughly explained by HÖRSTADIUS (1950), referring to cells migrating from the ectoderm.

It is very difficult to distinguish between endo- and ectomesenchyme in the teleostean and holostean material studied here. It may be noted, however, that the material was already fixed in BOUIN and SMITH, and the possibilities of using different staining methods hence reduced. In acipenserids and dipnoans the BOUIN fixation and the usual Azan-Mallory staining method have yet given quite satisfactory distinctions, reminding of those in selachians and amphibians. The ectomesenchyme is loaded with large yolk globules that may remain even in the early chondral stage (p. 208).

5. Seven stages—from 3.9 to 23 mm— of the *ontogeny* of the chondrocranium in *Hepsetus* have been described and reconstructed (chapter III).

Neural cranium

The characids seem to have vestigial *premandibular somites* (p. 211, Figs. 1, 7, s.l.). These are ventrally connected with the caudal end of the trabecles. No cells, however, are seen to detach from the somites to contribute to the formation of the trabecles. Instead they furnish material of the premandibular eye muscles, mm. obliquus inferior and rectus anterior, inferior and superior (p. 211).

The characidean *trabecles* may be of a primitive type comparable to that in *Acipenser* and *Amia*. As in these fishes they are initially connected with the polar cartilages first medially to the carotis interna, forming a carotid incissure (Fig. 2, c.fi.), and later also laterally to the carotid, constituting a carotid foramen (Fig. 7, fo.c.) (chapter III, IV). Only the lateral connection will, however, chondrify in *Hepsetus*, whereas in *Amia* just a part and in *Acipenser* the entire medial connection chondrifies (p. 298).

As in *Salmo* and *Leuciscus* among the teleosts investigated up to now *polar cartilages* are discernable also in *Hepsetus*. Like in the fishes mentioned they are situated between the connection of the carotis interna and the mandibular aorta (pseudobranchial artery), ventrally, and the m.rectus externus, dorsally (Figs. 1, 7, 18, pb.). Anteriorly and posteriorly the polar cartilage rudiments are only loosely connected with the trabecles and basiotic laminas, respectively.

As in sharks, *Salmo* and *Acipenser* the *otic cartilages* in *Hepsetus* are formed in two separate portions, one below the anterior and one below the posterior part of the otic vesicles. They later fuse laterally and chondrify in one piece.

The anterior otic cartilage (Fig. 2, ca.a.) is on each side medially continuous with the anterior basicapsular commissure (a.bc.c.). This forms in the facial region, whereas the basivestibular (bv.c.) and later the posterior basicapsular commissures (Fig. 13, p.bc.c.) are connected with the posterior otic cartilage in the glossopharyngeal and vagal regions, respectively. The posterior basicapsular commissures are formed from the posterior otic cartilages. In spite of the fact that the other two commissures are initially connected with the basiotic laminas the similar arrangement of the cells in the commissures and of those of the otic cartilages makes it probable, that even the anterior basicapsular and the basivestibular commissures are originally formed from the otic cartilages. Phylogenetically considered the otic cartilages have then formed independently in early characids or their predecessors like they do in *Acipenseridae* and *Selachii* to-day (p. 265).

Along the ventromedial margin of the otic vesicles the anterior, basivestibular and posterior commissures border the *basiotic laminas*. These are, as in *Salmo* and *Amia* (Holmgren 1943), rostrally connected with the polar cartilages and stretch back to the vagal region. They can all the time be discerned from the surrounding elements (p. 262, Fig. 34). However, they do not form in one or two portions as Holmgren stated for *Amia* and *Salmo* but in three somewhat thicker parts, the anterior, mesotic and occipital (Figs. 2, 8, l.bas.a., l.bas.m., l.bas.o.). These are regularly arranged in relation to the dorsal nerve roots and are primarily ventrolaterally connected with the mesoderm of the hyal arch and the first and second branchial arches (Fig. 2, s.3—5.m.). It is possible to distinguish histologically the anterior portion even after chondrification (p. 264).

Between the notochord and the thick basiotic laminas the thinner *parachordals* appear. They are also formed in three portions, regularly arranged as those of the basiotic laminas (pp. 262—263 Figs. 2, 8, pch.a., pch.m., pch.o.).

Dorsolaterally to the parachordals two pairs of arches arise. A *preoccipital arch* (Figs. 2, 8, 13, pr.o.) forms in connection with each mesotic parachordal and an *occipital arch* (p.o.) in connection with each occipital parachordal. For comparative anatomical reasons those arches should be neural arches (p. 263). The occipital arches develop strongly and form the main part of the posterior covering of the labyrinths (Figs. 6, 12, 15).

The *dorsal frame of the eyes* develops rather late (stage IV) and from com-

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paratively many chondrification centers. If compared with other actinopterygians, the *Hepsetus* type may be grouped together with Amia and the silurids (Fig. 24). Supposing that the complete skull roof type found in *Lepisosteus* (HAMMARBERG 1937) is primitive, *Hepsetus* has a type of skull roof, intermediate between the primitive *Lepisosteus* type and the secondary type, present in *Clupea, Salmo* and *Gasterosteus* (p. 241).

The trabecles are initially rostrally continuous with the mandibular arches. From this common mesenchyme, which is here called the anterior mesenchyme, the *trabecular commissure* (Fig. 16, 17, 23, tr.c.) and the large *planum ethmoidale* (pl.e.) will differentiate. *Lamina orbitonasalia* (Figs. 15, 25, l.o.n.) form behind the nasal sacs, initially only loosely connected with the lateral margin of the trabecular commissure. Further, the rudiments of the lamina orbitonasalia are dorsally free from or only loosely connected with the neural cranium, but have instead, ventrally, a broad connection with the processus pterygoideus of the palatoquadrates.

Visceral cranium

The visceral arches are primarly formed in the same way: a core of mesoderm surrounded by ectomesenchyme. In the inner part of this the cells multiply and the nuclei become round and regularly arranged (perpendicularly to the length of the arch). This inner blastemal portion is the future skeletal arch. The external, subepidermal portion of the ectomesenchyme has smaller and irregularly arranged nuclei, more stainable in Azan. From this mesenchyme, gill rays are formed in the branchial arches.

In the *mesotic* (branchial) *arches* the initially continuous skeletal arches later chondrify into different portions, the hypo-, cerato-, epi- and infrapharyngeal elements. The infrapharyngobranchials grow out from the epal elements, as do the suprapharyngobranchials. The latter ones, however, chondrify in connection with the epal elements and posteriorly to the branchial nerve and artery.

Five initially separate skeletal arches are formed in the characids. Contrary to other actinopterygians investigated so far a fused epi-suprapharyngeal element is present even in the fifth arch (Figs. 25, 31, 33, 38). This seems to be a very primitive character (p. 250).

The dorsal part of the subepidermal blastema, the dorsolateral blastema, is densely nucleified. From this portion dorsal gill rays differentiate (Fig. 35, g.r.), situated on the epibranchials in the roof of the pharynx.

The prootic arches have no real gills. Yet there is formed a subepidermal blastema. However, most of it later vanishes or differentiates into connective tissue. In the hyal arch the subepidermal blastema forms partly the lateral portion of the stylohyal, and partly that part of the hyomandibula situated above the foramen hyomandibularis, the laterohyal (Figs. 1, 3, 4, l.hy.). The latter is homologous with the dorsolateral blastemas of the branchial arches. In

the mandibular arch the dorsolateral blastema constitutes a vestige which—according to the position—should be homologous with the processus oticus externus in selachians (p. 214). However, only the base chondrifies (Figs. 4, 6, 7, 12, 15, pr.ot.e.), whereas the rest vanishes.

6. Embryologic comparisons with other teleosts and with *Amia, Lepisosteus, Acipenser, Polypterus, Squalus* and dipnoans as well as results of literature studies have been accounted for in chapter IV and V. It is obvious that in most of the characters mentioned above the characidean chondrocranium shows such primitive characters that the characids in these respects should be placed at the base of the *Teleostei.*

In this connection it should be pointed out that SAGEMEHL (1885) has in the characidean osteocranium traced similarities with *Amia,* for which reason he suggested that the characids have originated from *Amia.* Other authors, on the contrary, considered the similarities to be results of a converged evolution and instead pointed to certain dissimilarities in teeth, scales and viscera (p. 204).

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The present investigation of *Hepsetus* has certainly revealed specialisations in comparison with *Amia:* a large ethmoidal plate (Figs. 16, 23, 26, 34, pl.e.), no pila lateralis, a processus posterior of the palatoquadrate (Figs. 12, 15, 33, pr.p.), sometimes separate submaxillary cartilage(s) (Fig. 84, s.mx.c.), a prelingual cartilage (Figs. 33, 39, pl.c.), a secondary division of the basihyal (b.hy.$_{1-2}$). But there are also similarities with *Amia:* the formation of preoccipital processes (Figs. 2, 8, 14, 16, pr.o.), lateral commissures (Figs. 13, 16, 26, l.c.), a posterior myodome with only m. recti externi (Fig. 34, p.my.), the dorsal frame of the eye (Fig. 24), lamina orbitonasalia (Figs. 15, 25, 33, l.o.n.), basal (Figs. 1, 6, 12, 15, 25, 33, pr.bas.) and oticus internus processes (Figs. 25, 33, pr.ot.), an intermediating body of the pterygoid process (Figs. 13, 16, 17, 23, 26, int.b.) and a primarily complete hyal aorta (Fig. 10, ao.hy.). Many of these characters or the development of them have been described also in other actinopterygians, but so far they have not all been found in one single group, as they have in characids.

Concerning certain characters, however, the characids (*Hepsetus*) seem, to be even more primitive than *Amia*: a more distinct segmentation of the cranial base (Figs. 2, 8), an almost complete fifth branhial arch (Figs. 20, 30, 38), four separate infrapharyngobranchials (Fig. 39, i.b.$_{1-4}$) and a vestigial processus oticus externus (Figs. 1, 6, 12, pr.ot.e.).

This similarity with *Amia* and the presence of even more primitive characters in *Characidae* make it necessary to accept WESTOLL's (1944) criticism of the division of the higher actinopterygians into *Holostei* and *Teleostei* (cf. STENSIÖ 1932, NIELSEN 1949, OLSSON 1958, BERG 1958, JARVIK 1959). "It seems far better to regard *all* Actinopterygii which notably differ from palaeoniscids in structure as forming a fringe of evolutionary twigs and branches around a central compact bush in which we may hope some day to distinguish the origin of each derivative group. Within this fringe it is legitimate to recognize close and similar groups, to

which handy names such as Teleostei may be given but of group names now in use neither Holostei nor Sub-Holostei can be considered satisfactory. The former certainly covers polyphyletic forms . . .". WESTOLL's opinion appears to be fully justified when applied to the phyletic relations between *Amia* and *Characidae* related above.

7. The composition and origin of the *chondrocranial base* has been investigated in different representatives of the gnathostomian fishes (chapter IV).

The *notochord* is the first element to form. It usually extends to the hypophysis but is later resorbed anteriorly.

Up to now the opinion has prevailed that separate *parachordals* and *basiotic laminas* are present only in selachians and higher actinopterygians (cf. HOLMGREN 1943). As might be expected, however, parachordals and basiotic laminas are found also in recent lower actinopterygians (*Acipenser* and *Polypterus*) and in dipnoans (pp. 273, 274, 279). Except in *Salmo* (HOLMGREN) and *Hepsetus* (chapter III) they have, among the teleosts, been traced also in *Esox* and *Heterotis* (p. 267).

As to the development of the actinopterygian and dipnoan parachordals and basiotic laminas the present investigation has shown (1) that they are not formed from a somitic mesoderm as in selachians but from an unsegmented mesoderm that later turns into endomesenchyme. Yet the parachordals and laminas may initially be constituted of three portions, the anterior, mesotic and occipital (cf. above). The laminar portions are laterally continuous with the lateral plate mesoderm of the hyal and first and second branchial arches. In selachians they are primarily continuous with the mesoderm of only the hyal and first branchial arches (HOLMGREN 1940). The laminar portions are often (cf. *Hepsetus*) situated in a regular position to the dorsal nerve roots. The basiotic laminas in actinopterygians and dipnoans are thus initially divided in a way comparable to that of the selachian laminar rudiments. This may indicate that there are traces of an original segmentation of the otic region of the actinopterygian and dipnoan head (up to the hypophysis), a segmentation, however, that applies only to the endomesenchyme, not to the mesoderm as in selachians. The actinopterygian and dipnoan otic "somites" are in this paper called "mesenchyme somites" (p. 280). (2) If this theory is correct the anterior parachordals and basiotic laminas should represent the 3rd (hyal) somites (Fig. 43), the mesotic parachordals and laminas the 4th (lst branchial or metotic) somites and the occipital parachordals and laminas the 5th (2nd branchial or metotic) somites. (3) If compared with the development of the selachian parachordals and supposing that the occipital and preoccipital arches represent neural arches (p. 263), it seems reasonable to suggest also the actinopterygian and dipnoan parachordals (formed of endomesenchyme and situated close to the notochord) to represent sclerotomic mesenchyme (p. 268). (4) It is usually possible to discern the basiotic laminas even after their chondrification, because the chondrocytes are arranged differently than in the

parachordals and since the chondrocytes in the demarcation lines are separated by less intercellular substance. (5) For the same reasons even the chondrified anterior basiotic laminas may be delimited from the mesotic and occipital (*Hepsetus*). (6) The basiotic laminas have the same thickness as the parachordals in lower actinopterygians and dipnoans, whereas the laminas dominate in higher actinopterygians. (7) The position of the basiotic laminas laterally to the parachordals (sclerotomes) and their posterior connection with the occipital dermomyotomes (Figs. 45, 49) indicate that the basiotic laminas might represent transformed dermo-myotomes of the otic region. An investigation of early embryos of *Squalus acanthias* strengthens this theory (pp. 282—284, Figs. 56—62). The sclerotomes of the hyal and first branchial somites form the anterior portion of the parachordals, whereas parts of the dermo-myotomes of the same somites form the two basiotic laminar portions (Figs. 43, 56—62). The rest of the dermatomes form connective tissue and the anterior part of the hyal myotome differentiates into the external rectus muscle.

This theory shows that the fate of the mesodermal somites in the head is not fixed definitely. Such an opinion has, however, already been suggested both for other embryological and for palaeontological reasons (p. 286).

The *otic cartilages* are in *Acipenser* (Figs. 45—47), *Salmo* (Fig. 41) and *Hepsetus* (Figs. 2, 8) formed in two independent portions, one below the anterior and one below the posterior part of the otic vesicles. The anterior portions develop faster than the posterior ones. As to the otic commissures reference can be made to *Hepsetus* (p. 348).

Polar cartilages have been found in *Hepsetus* (p. 348). Neither in *Hepsetus* nor in any other higher actinopterygian described or investigated here, it has been possible to settle their origin with any certainty. As there are different opinions concerning their origin even in fishes with a distinct segmentation of the prootic mesoderm, *Acipenser* and *Dipnoi* have been investigated here. A detailed description of the ontogeny and morphological relations of the polar cartilages in *Acipenser* is given on pp. 290—291. It shows that cells of the polar cartilages together with cells of the acrochordal tissue proliferate from the medial wall of the mandibular somites alone (Figs. 45, 63). Consequently the polar cartilages and acrochordal tissue, as is the case in selachians (Holmgren 1940), seem to represent the mandibular sclerotomes. The only essential difference between acipenserids and selachians in this respect seems to be that the cells of the selachian acrochordal tissue proliferate much earlier than those of the polar cartilages.

No separate polar cartilages have been found in *Dipnoi* (p. 292). Because of comparative anatomical reasons, however, it seems probable that they are incorporated in the cranial base. In *Neoceratodus* (Figs. 49, 50) they are caudally continuous with the parachordals, in *Protopterus* and *Lepidosiren* with the basiotic laminas (Fig. 53).

The literature concerning the *trabecles* is discussed on pp. 293—297. It is found

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that the experimental and anatomical embryologists actually do not give a plain answer to the question of the origin of the trabecles. In the present investigation it is certainly shown that the trabecles in teleosts (*Salmo, Hepsetus, Esox*), *Acipenser* and *Dipnoi* are caudally bordered by the premandibular somites, but also that in *Acipenser* and *Dipnoi* they are *entirely* ectomesenchymal (pp. 300, 301). Provided that the amphibian trabecles also are entirely ectomesenchymal (p. 297) and provided that they in *Agnatha* and *Selachii* (p. 293) really can be shown to be clearly dual as to their origin, there should exist two types of trabecles, one formed only of ectomesenchyme (*Teleostomi, Dipnoi, Amphibia*) and one type consisting of both endo- and ectomesenchyme (*Agnatha, Elasmobranchii)* (p. 302).

As to the connection between the actinopterygian trabecles and polar cartilages four types have been shown to exist (p. 298).

The problem of the trabecles being transformed visceral elements are discussed on pp. 302—303. The embryological and palaeontological evidences for a visceral origin seem to be more important than the evidences of a somitic origin.

8. The *composition of the visceral cranium* in fishes is discussed in chapter V.

The *mesotic* (branchial) *arches* give the basis for understanding the prootic arches. Among the teleostomes they are primarily composed not of four but of five elements, the infrapharyngo-, suprapharyngo-, epi-, cerato-, and hypobranchial. In some lower and in all higher teleostomes investigated so far, the suprapharyngobranchials do not separate from the epal elements but constitute a caudodorsal process of the latter ones caudally to the visceral aorta and nerve. In the posterior branchial arches, reductions of the peripheral parts are very common. *Hepsetus* seems to be primitive in this respect, because a fused epi-suprapharyngobranchial$_5$ is always present (cf. above).

The ontogeny of the branchial arches in *Acipenser* and *Hepsetus* principally agrees. The infra- and suprapharyngobranchials develop from the epal elements. In *Acipenser,* however, the suprapharyngobranchials reach the neural skull and segment off. Of special interest concerning the development of the branchial arches in *Hepsetus* are the dorsolateral blastemas (p. 217, 349).

The *prootic arches* are usually considered to be represented by the hyal and mandibular arches. This investigation, however, has justified the theory of a vestigial premandibular arch in addition.

In the *hyal arch* the interest has been concentrated to the hyomandibula, the symplectic and the stylohyal.

The ontogeny of the hyomandibula in *Neoceratodus* has been described (pp. 309—315, Figs. 72—75) and remarks have been made on the ontogeny of the actinopterygian hyomandibula (pp. 315—324, Figs. 3, 4, 6, 12, 15, 71—81, 81—86). The general conclusions are summarized on p. 326.

The hyomandibula in *Neoceratodus* and actinopterygians seems to agree in principal. Primarily it seems to consist of an epal and a fused infra-suprapharyn-

geal element as well as of a dorsal remainder of the subepidermal blastema, the laterohyal; this is connected with the dorsolateral margin of the epihyal and homologous with the gill ray producing dorsolateral blastemas of the branchial arches (cf. p. 349). In *Neoceratodus* the infra-suprapharyngohyal chondrifies independently from the epal element, detaches from it and instead joins the neural cranium in a position comparable to that of the lateral commissure in actinopterygians. These structures are therefore probably homologous. If that is the case the first stages in the ontogeny of the actinopterygian infra-suprapharyngohyal (lateral commissure) has been lost. This is accordingly an example of abbreviation in the ontogeny of the actinopterygian hyal arch.

The laterohyal in *Neoceratodus* chondrifies independently or in connection with the epihyal. Caudally it develops a small opercular process which often chondrifies separately. The tr.hyomandibularis facialis runs anterior to the laterohyal. In teleostomians the laterohyal is of a different size, from a small vestige in *Polypterus* and *Acipenser* to a large part of the hyomandibula in higher actinopterygians (?and crossopterygians) situated above the foramen hyomandibularis and constituting the anterior and posterior articular heads and the opercular process.

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Indications of how the dorsal hyal gill rays have transformed into the laterohyal is discussed in pp. 325—327. Parallels seem to be present in the branchial arches in *Polypterus* and in the selachian hyal arch (the dorsal extrahyal in sharks and the pseudohyoid in rays).

Comparisons are made with the selachian and holocephalian hyomandibula (pp. 308, 328). *Neoceratodus* and the holocephalians have a hyomandibula, in which primarily all elements are present. In the holocephalians, however, these remain also at the adult stage, whereas in *Neoceratodus* the fused pharyngeal elements detach and constitute the lateral commissure. The rays with their epihyal and pseudohyoid seem to have a hyomandibula principally homologous with the adult hyomandibula in *Neoceratodus* and teleostomians (epi- and laterohyal), whereas the sharks are said to have also a suprapharyngeal element fused to the epihyal.

The problem why the hyomandibula articulates ventrally to the jugular vein in selachians but dorsally to it in teleostomes is discussed on pp. 328—331. In the present investigation it has been found that the position of the suprapharyngobranchials in relation to the jugular vein is not fixed, as many authors have suggested. The different relations of the selachian and telostomian hyal arch in relation to the jugular vein can therefore not a priori be considered as a fundamental difference. The difference might, however, be explained by the theory that the teleostomian hyomandibula articulates with the neural cranium by means of transformed hyal gill rays (Fig. 81), not by means of a suprapharyngohyal (SCHMALHAUSEN and others) or a suprapharyngohyal + transformed hyal gill rays (HOLMGREN).

The homology of the actinopterygian *symplectic* is discussed on pp. 331—332. It is shown that this cartilage is primarily an anteroventral process of the hyomandibula (Figs. 1, 6, 76—81), but that it secondarily may detach (Fig. 12) and articulate or get in contact with the mandibular arch. This is, however, probably an adaptation related to the actinopterygian respiration type, and not an indication of an earlier existence as a mandibular ray. The single or double mandibular rays present in selachians (HOLMGREN 1940, 1943) should accordingly not be homologous with the actinopterygian symplectic.

Another element characteristic of the actinopterygian hyal arch is the *stylohyal*. The present investigation of *Neoceratodus* (p. 310, Fig. 71) and *Hepsetus* (p. 349) has given indications that HOLMGREN's hyal ray theory may be correct (p. 333).

The possibility of finding typical visceral arch elements in the *mandibular arch* is discussed on pp. 333—340. The theory that a hypal and basal element is present in recent fishes is criticized. More investigations are wanted in this matter. For comparative anatomical reasons the lower jaw in *Hepsetus* may represent a *ceratomandibula* (Fig. 85, c.md.) and the large dorsorostrally pointing coronoid process a *stylomandibula* (st.md.).

The regular serial arrangement of the upper pharyngeal dental plates in *Eusthenopteron* caused JARVIK (1954) to suggest that "in *Eusthenopteron* and other gnathostomes there are three prootic visceral arches ... Each of these arches, like the branchial arches, consists dorsally of infrapharyngeal, suprapharyngeal and epal elements ... The main modifications consist of fusions with the neighbouring elements of the same or adjoining arches or with the neural endocranium". JARVIK's theory seems to be valid also for *Hepsetus*, because even here there are traces of a premandibular arch (Fig. 85). As in the hyal arch there are also in the mandibular and premandibular arches traces of pharyngeal elements. The main conclusions drawn in this respect are demonstrated in Fig. 85. They apply in the first place to the conditions in *Hepsetus* but comparisons are also made with other fishes.

The *epimandibula* (e.md.) is represented by the pars quadrata and its ventrorostral part, the *mandibulosymplectic* (md.sy.), is for comparative anatomical reasons possibly homologous with the hyalosymplectic. The *infrapharyngomandibula* (i.md.) has probably been incorporated in the neural cranium, where it forms all or part of the trabecle (pp. 302, 353). Supposing that the processus oticus internus is homologous with that of the selachians and that HOLMGREN's theory of its gill ray origin is correct, the *suprapharyngomandibula* is missing. In *Dipnoi* there is a processus ascendens and in *Eusthenopteron* JARVIK has homologized processus ascendens with a suprapharyngomandibula. It is, however, not clear if the two structures are homologous. In *Hepsetus* there is initially also a processus oticus externus, which because of its position is homologous with the dorsolateral blastemas in the branchial arches (cf. above), and thus should repre-

sent vestigial *mandibular gill rays*. In adult stages the processus oticus externus has been incorporated in the pars quadrata.

The *epipremandibula* (e.pmd.) may be represented by the processus pterygoideus of the palatoquadrate (p. 342). The *infrapharyngopremandibula* (i. pmd.) seems to be represented by half the palatoquadrate commissure covering the ventral tip of the ethmoidal plate. Later it partially fuses with the ethmoidal plate, partially forms the rudiments of the premaxillary, the vomer and the endopterygoid. The tip of the pterygoid process is formed by the intermediating body. As in *Acipenser, Amia* and *Salmo* this seems to be a secondary formation and is in other characids detached as the submaxillary cartilage(s) (pp. 342—344, Figs. 17, 37, 84). The *suprapharyngopremandibula* (Fig. 85, s.pmd.), finally, is probably represented by the lamina orbitonasalis and becomes hence also fused with the neural cranium (p. 346).

Thus, according to this theory the palatoquadrate in actinopterygians is a complex structure belonging to two visceral arches. In addition it includes even vestiges of mandibular (spiracular) gill rays (processus oticus internus).

With this theory as a basis the conditions in selachians and dipnoans are discussed. Contrary to the conditions in teleostomes the selachian infrapharyngopremandibula (pars palatina) constitutes part of the palatoquadrate, whereas the epipremandibula seems to be lost or embodied into either of these elements. The suprapharyngopremandibula probably does not correspond to the processus orbitalis but is, as in teleostomes, represented by the lamina orbitonasalis (p. 346). In *Neoceratodus* the processus pterygoideus is reduced (Fig. 72) and therefore also the epipremandibula. It seems, however, to be present in the blastemal stage, and so are also the pharyngeal elements. The suprapharyngopremandibula even chondrifies (lamina orbitonasalis). The mandibular arch is certainly strongly developed and adaptated, but its fusion with the caudal end of the trabecle (infrapharyngomandibula) may be primitive. Further, it has a large processus ascendens that possibly represents a suprapharyngomandibula (cf. above).

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Abbreviations

a.ah. anterior articular head of hyomandibula.
a.bc.c. anterior basicapsular commissure.
a.d. articular disc of hyomandibula.
a.hy. articulation for hyomandibula.
a.hy.a. afferent hyal artery.
a.m. arteria mandibularis.
a.mes. anterior mesenchyme.
a.md. articulation for mandibula.
a.my. anterior myodome.
a.oph. arteria ophthalmicus.
a.opt. arteria opticus.
a.ot.pr. anterior otic process.
a.pal.bl. autopalatine blastema.
a.ps.a. afferent pseudobranchial artery.
a.b.a. afferent branchial artery.
acr. acrochordal tissue
ang. angular.
ang.bl. angular blastema.
ao.b. aorta branchialis.
ao.d. aorta dorsalis.
ao.hy. aorta hyoideus.
ao.hy.v. ventral part of aorta hyoideus.
ao.md. aorta mandibularis.
b.b. basibranchiale.
b.c. basis cranii.
b.hy. basihyale.
br.a. branchial arch.
bv.c. basivestibular commissure.
c.b. ceratobranchial.
c.c. carotid commissure.
c.fi. carotid incissure.
c.fo. carotid foramen.
c.hy. ceratohyal.
c.i. carotis interna.
c.on. cavum orbitonasale.
c.po. crista parotica.
c.se. cartilago supraentethmoidalis.
c.so.a. cartilago supraorbitalis anterior.
c.so.p. cartilago supraorbitalis posterior.
c.w. cranial wall.
ca. otic cartilage.
ca.a. anterior part of otic cartilage.
ca.p. posterior part of otic cartilage.
ca.v. ventral part of otic cartilage.
ch. notochord.
co.br.d. musculus constrictor branchialis dorsalis.
co.hy.d. musculus constrictor hyoideus dorsalis.
co.hy.v. musculus constrictor hyoideus ventralis.
co.md. musculus constrictor mandibularis.
co.md.d. musculus constrictor mandibularis dorsalis.
d. dermatome.
d.-my. dermo-myotome.
d.pal.bl. dermopalatine blastema.
de. dentary
de.bl. dentary blastema.
e.b. epibranchial.
ec.pt. ectopterygoid.
e.hy. epihyal.
e.md. epimandibula.
e.pm. epipremandibula.
e.ps.a. efferent pseudobranchial artery.
e.s. eye stalk.
eff.hy. efferent hyal artery.
en.pt. entopterygoid.
ep.b. epiphysial bar.
ep.c. epiphysial cartilage.
f.b. fenestra basicranialis.
f.o.a. foramen olfactorium advehens.
f.o.e. foramen olfactorium evehens.
fe.b. fenestra basicapsularis.
fo.c. foramen carotis.

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fo.hy. foramen hyomandibularis.
fo.f. foramen facialis.
fo.n. fossa nasalis.
fo.pq. foramen palatoquadratum.
g.V. ganglion trigeminus.
g.VII. ganglion facialis.
g.IX. ganglion glossopharyngeus.
g.X. ganglion vagus.
g.r. gill ray.
h.b. hypobranchial.
h.hy. hypohyal.
hy. hyomandibula
hyp. hypophysis.
i.b. infrapharyngobranchial.
i.hy. infrapharyngohyal.
i.-s.hy. infrapharyngo-suprapharyngohyal.
i.md. infrapharyngomandibula.
i.o.s. interorbital septum.
i.pm. infrapharyngopremandibula.
inf. infundibulum.
int.b. intermediating body of palatoquadrate.
io.v. infraorbital vein.
j.v. (jug.v.) jugular vein.
l.bas. lamina basiotica.
l.bas.a. lamina basiotica anterior.
l.bas.m. lamina basiotica mesotica.
l.bas.o. lamina basiotica occipitalis.
l.br. laterobranchial (dorsolateral part of branchial arch).
l.c. lateral commissure.
"l.c." lateral commissure after ossification.
l.hy. laterohyal (dorsolateral part of hyal arch).
l.o.n. lamina orbitonasalis.
l.p. lateral plate mesoderm.
m.a.h. musculus adductor hyomandibulae.
m. add. musculus adductor mandibulae.
M.c. meckelian cartilage.
m.l.h. musculus levator hyomandibulae.
m.lev.a.p. musculus levator arcus palatinus.
m.lev. musculus levator branchialis.
m.o.i. musculus obliquus inferior.
m.o.s. musculus obliquus superior.
m.r.a. musculus rectus anterior.
m.r.e. musculus rectus externus.
m.r.h. musculus retractor hyomandibulae.
m.r.s. musculus rectus superior.
md. mandible.
md.a. mandibular arch.
mx. maxillary.
my. myotome.
na.s. nasal sac.
o.a. orbital artery.
o.w. part of the orbital wall.
on.c. canal in lamina orbitonasalis.
op. operculum.
ot.p. otic placode.
ot.v. otic vesicle.
p.a. pila antotica.
p.a.h. posterior articular head of hyomandibula.
p.b. polar cartilage.
p.bc.c. posterior basicapsular commissure.
p.c.v. posterior cerebral vein.
p.l. pila lateralis.
p.mx. premaxillary.
p.my. posterior myodome
p.o. pila occipitalia.
p.occ. basioccipital bone
p.ot.pr. postotic process.
pa.b. paraphysial bar.
pa.qu. pars quadrata.
pch. parachordal.
pch.a. anterior parachordal.
pch.m. mesotic parachordal.
pch.o. occipital parachordal.
ph. pharynx.
pl.c. prelingual cartilage.
pl.e. planum ethmoidale.
po.b. prootic bridge.
po.r. preoptic root.
pr.bas. processus basalis.
pr.co. processus coronoideus.
pr.en. processus entethmoidalis.
pr.hy. musculus protractor hyomandibulae.
pr.o. processus preoccipitalis.
pr.op. processus opercularis.
pr.orb. processus orbitalis.
pr.ot. processus oticus (if obliquely lined, prootic bone).
pr.ot.e. processus oticus externus.
pr.ot.i. processus oticus internus.
pr.p. processus posterior.
pr.p.pal. processus postpalatinus.
pr.po. processus postorbitalis
pr.p.ot. processus prooticus.
pr.pt. processus pterygoideus.
pr.pt.p. processus pterygoideus proper.
pr.ra. processus retroarticularis.

ps. pseudobranchie.
pto. pterotic.
s.1—5. somite 1 to 5.
s.l.com. premandibular somite commissure.
s.1—5.m. muscle process of first to fifth somite.
s.b. suprapharyngobranchial.
s.bl. symphysial blastema of mandibles.
s.d. sucking disc.
s.hy. suprapharyngohyal.
s.mx.c. submaxillary cartilage.
s.n. septum nasi.
s.pm. suprapharyngopremandibula.
sc.m. sclerotic membrane.
scl. sclerotome.
sec.v. secondary vein.
st.hy. stylohyal.
st.md. stylomandibula.
sy. symplectic.
t.m.a. taenia marginalis anterior.
t.m.e. taenia marginalis ethmoidalis.
t.m.p. taenia marginalis posterior.
t.m.po. taenia marginalis postorbitalis.
t.me.a. taenia medialis anterior.
t.me.p. taenia medialis posterior.
t.o. tectum synoticum.
tr. trabecle.
tr.c. trabecular commissure.
tr.com. trabecula communis.
tr.e. lateral part of trabecle.
tr.hy.VII truncus hyomandibularis facialis.
tr.i. medial part of trabecle.
v. vertebra.
V.1. nervus ophthalmicus trigeminus.
V.2. nervus maxillaris trigeminus.
V.3. nervus mandibularis trigeminus.
VII.md. nervus mandibularis facialis
VII.hy. nervus hyomandibularis facialis
VII.p. nervus palatinus facialis
IX nervus glossopharyngeus.
IX.pr. nervus glossopharyngeus pretrematicus.
IX.pt. nervus glossopharyngeus posttrematicus.
X.1—4 nervus vagus, four rami.
X.1—3.pr. nervus vagus, pretrematic branch of the rami.
X.1—3.pt. nervus vagus posttrematic branch of the rami.
X.int. nervus vagus, ramus intestinalis.
X.lat. nervus vagus, ramus lateralis.

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The secretory conditions in the praeoptico-hypophysial system of the roach *(Leuciscus rutilus)* and during the histogenesis

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Introduction

The histogenesis of the diencephalic neurosecretory system has in the last few years been subjected to studies in several vertebrate classes (HILD 1951, BARGMANN et al. 1950, WINGSTRAND 1953, MAZZI 1954, DIEPEN et al. 1954 and RODECK and CAESAR 1956). According to the observations in these works the neurosecretory substances appear first in the neurohypophysis during the histogenesis and more or less later in the cell bodies. WINGSTRAND (1953) noted the simultaneous occurrence of the secretion in the proximal and distal regions of the system in the chick embryo.

The recent knowledge of the histogenesis of the praeoptico-hypophysial system in teleosts is based on observations by AZZALI (1952) and ARVY et al. (1956). HILD (1950) observed that the praeoptic nuclei and the neurohypophysis in *Tinca vulgaris* were poor in secretion at 40 mm length and the tracts difficult to trace. In the eel according to AZZALI the praeoptic nuclei seem to start their secretory

activity at a length of about 100 mm. The observations of ARVY et al. are worth special attention because these authors observed the first secretion in nucleus praeopticus three days after hatching but not until five days later in the neurohypophysis. This is in contrast to AZZALI's results with the late appearance of the secretion in the system and further to the observations in other vertebrate classes characterized by the first detectable secretion in the neurohypophysis.

The aim of the present study to determine the stage at which the system starts its activity in the roach (*Leuciscus rutilus*) was facilitated by the new histochemical methods worked out for a selective staining of the secretion (ADAMS and SLOPER 1956, MÜLLER 1957, cf. OLSSON 1958) and which were used in addition to the chrome haematoxylin method according to GOMORI (1941).

The histogenetic studies were preceded by an analysis of the secretory process in the adult specimens in order to get information about the morphology of the process which in teleosts may show considerable variations between the species (SCHARRER and SCHARRER 1954).

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Material and methods

The material was 14 adult roaches (*Leuciscus rutilus*) which were decapitated and the brains quickly dissected free and fixed in Bouin's mixture by immersion. The brains were cut in series sagittally and transversally in 5—7 μ sections and stained in chrome alum haematoxylin (GOMORI 1941), in Alcian Blue (ADAMS and SLOPER 1956) and in Astrablau with carmalum as counterstain according to OLSSON (1958). Alternate slides of two series were stained according to GOMORI (1941) and to ADAMS and SLOPER (1956). One brain was fixed in Carnoy's mixture (6 : 3 : 1) and stained by the methyl green-pyronin method (Brachet-Pappenheim) according to ABOLINS (1952). Ribonucleas (crystalline) 3 × of NBC in a concentration of 1 : 50,000 suited the purpose.

To study the histogenesis of the praeoptico-hypophysial system roach-eggs were artificially fertilized and they hatched after 13—14 days at 11°C. After hatching the spawns were kept in aquariums at room temperature. Out of this material fixed at regular intervals in Bouin's fluid specimens were chosen between the neurula stage and fishes 70 mm in standard length. Sagittal and transversal series were cut in 3—5 μ sections and stained in chrome alum haematoxylin (GOMORI 1941). The most critical stages just before and after hatching and between 50—70 mm in length were controlled with the Astrablau method (OLSSON 1958). Totally 59 specimens were investigated, 23 of which were stained according to the latter method.

All objects were imbedded in paraffin via methylbenzoat-celloidin (ROMEIS 1948, § 392).

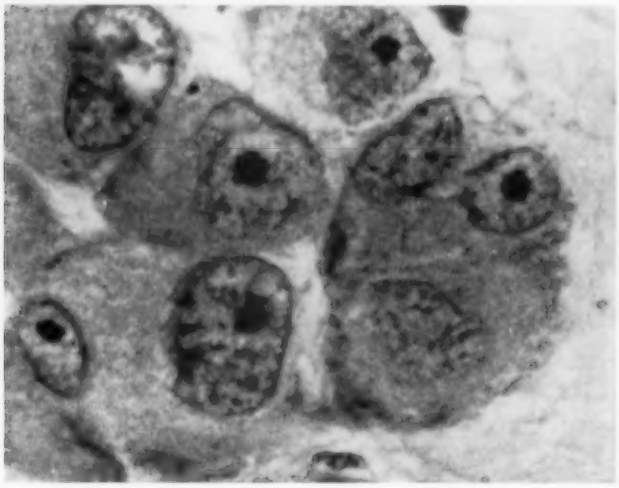

Fig. 1. Cells of nucleus praeopticus magnocellularis which except for a single droplet seem empty of secretion. (Sag. 6 μ, Cr.-Htx., 1 500 ×)

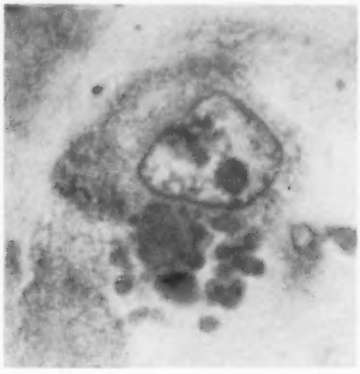

Fig. 2. A nucleus tuberis lateralis cell with phloxinophil droplets. (Sag. 6 μ, Cr.-Htx., 1 500 ×)

The secretory conditions in the praeoptico-hypophysial system of the adult roach

Nucleus praeopticus. The general structural plan of the praeoptico-hypophysial system of *Leuciscus rutilus* is in agreement with the descriptions of this system in teleosts which have been given by several authors (Palay 1945, Bargmann and Hild 1949, Hild 1950, Azzali 1952, Bargmann 1953, Diepen 1954 and Stahl 1956).

In the Gomori preparations the praeoptic cells have a basiphil reaction which

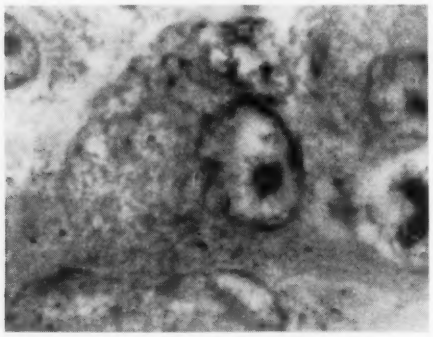

Fig. 3. A praeoptic cell with scattered granules in the cytoplasm. (Sag. 6 μ, Cr.-Htx., 1 500 ×)

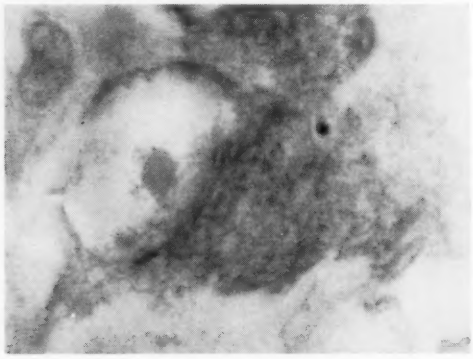

Fig. 4. A praeoptic cell displaying a secretory activity characterized by a homogenous positive reaction of the cytoplasm to the performic acid-alcian blue method according to Adams and Sloper. (Sag. 7 μ. 1 500 ×)

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is more distinct at the periphery of the cell bodies and in the nuclei-invaginations (Fig. 1). The nucleus tuberis lateralis cells have similar basiphil conditions and the cytoplasm as in the praeoptic cells has a faint blue hue (Fig. 2). The enzymatic method according to ABOLINS (1952) demonstrated the large concentrations of the ribonucleic acid in these distinct basiphil areas and further the occurrence of the ribonucleic acid homogenously distributed in the plasm in minute concentrations (cf. STAHL 1956). In the specimens examined on Gomori slides nucleus praeopticus seems rather inactive considering that most cells are lacking in secretion in the form of dark blue stained substances. However some cells contain solitary droplets (Fig. 1) and others finer cytoplasmatic granules stained by chrome haematoxylin (Fig. 3). In the latter cells "minimum-granules" are scattered sometimes tending to unit into larger particles. In the nucleus tuberis lateralis cells on the contrary the secretion occurs as larger droplets in the cell body taking phloxine but rejecting chrome haematoxylin, Astrablau and Alcian Blue

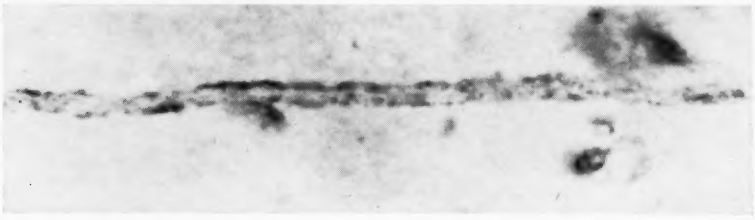

Fig. 5. A praeoptic axon with the secretion peripherically distributed (Sag. 6 μ, Cr.-Htx., 1 500 ×)

(Fig. 2). Thus the Gomori method (1941) gives the impression that nucleus praeopticus in several specimens almost lacks secretory material like the supraoptic and paraventricular nuclei in rabbits (Goslar 1952) in rats (Ortmann 1951) and in guinea-pigs (Rodeck and Caesar 1956). However the slides stained in Alcian Blue and Astrablau indicate that almost every cell in nucleus praeopticus contains secretory substances. Besides the granules and the droplets the cytoplasm has a blue tone of colour which in intensity shows variations between the cells (Fig. 4). The areas which in Gomori preparations are distinguished by a stronger basiphil reaction and the nucleoli take the counterstain carmalum. The positive reaction to these histochemical methods which does not affect the tuberis lateralis cells indicates that the secretory material occurs in a form homogenously dispersed in the cytoplasm and which is not apprehended as granules or droplets and further not recognizable on the Gomori slides.

Tractus praeoptico-hypophyseus. In spite of the striking poorness of secretion in nucleus praeopticus in the Gomori series the tracts appear rich in secretory material on the same slides. The secretion within tractus shows characteristic features in different parts of its course. In the proximal region from nucleus praeopticus to the chiasma ridge the widely scattered axons have a larger diameter than in the distal parts and a fine granulation appears in the axons especially in the immediate vicinity of the axolemma (Fig. 5). This dark blue stained material varies in size and form from almost a submicroscopical level to larger irregular particles, granules and droplets. These peripheral "Gomori-positive" substances have their counterparts on the Astrablau and Alcian Blue slides and cannot be considered artefacts caused by the Gomori method (cf. Goslar 1952, Goslar and Tischendorf 1953, 1955). Very frequently these parts of the axons have larger droplets as seen in Fig. 6 and more rarely do smaller droplets occur in great numbers which may completely fill the axon (Fig. 7). The larger droplets are the predominant form of secretion in these regions. The fibres descending to enter the tuber region and still more distally where they pass nucleus tuberis lateralis show a diminished diameter and often have a beaded appearence and are homogenously filled with secretion.

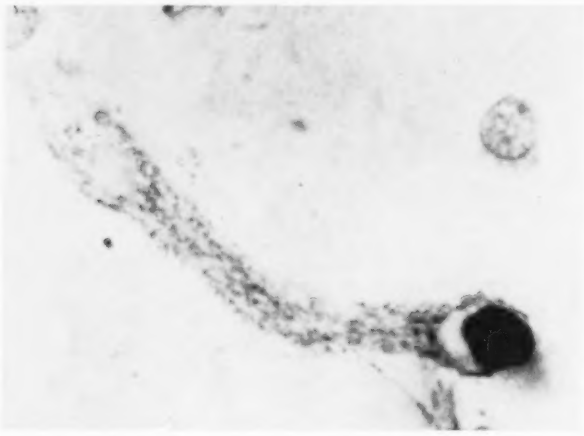

Fig. 6. A praeoptic axon in the proximal region with peripherically arranged secretory material, which could be supposed to accumulate and fuse to form larger droplets. (Sag. 7 μ, Cr.-Htx., 1 500 ×)

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Fig. 7. The proximal part of a praeoptic axon filled with smaller droplets. (Sag. 6 μ, Astrablau 1 500 ×)

Neurohypophysis. The conditions in the infundibular region and within the pituitary are quite in accordance with the opinions of HILD (1950) and BARGMANN (1953), and this is valid for the histochemical preparations as well. In infundibulum large Herring bodies are seen in the usual form completely or peripherically occupied by material stained dark blue by chrome haematoxylin and the hypophysial parts of the axons are homogenously stained forming an irregular granular pattern around the vessels. Even in this case it has not been possible to establish whether the granules may split off or are always parts of the axons.

The conditions described vary within small limits. It does occur, however, that the axons are filled and have beads even in the vicinity of nucleus praeopticus but on the other hand larger solitary droplets typical of the proximal parts could not be observed in the infundibular region.

Discussion. The comparative study of the Alcian Blue and the Astrablau series with the Gomori slides gives valuable information about the secretory process. Cells of the praeoptic and tuberis lateralis nuclei which seem empty of secretion

have the same reaction on the Gomori slides but differ in the histochemical preparations. The former cells have a blue reaction quite absent in the latter. Therefore it seems very likely that the praeoptic cells have secretion almost homogenously distributed in the cytoplasm and that the difficulties in distinguishing it on the Gomori slides are caused by the basiphil properties of the plasm also common to the tuberis lateralis cells. A distinct positive reaction of the Gomori method as expressed by RODECK and CAESAR (1956) depends on the concentration of the secretion. First when the secretion forms aggregates measuring more than 200 mμ or occurs in larger concentrations it will be observed as a substance reacting positively to chrome haematoxylin.

HANSTRÖM paid special attention to the marked peripherical positions of the secretion in the proximal parts of the axons from the diencephalic neurosecretory cells which he had described in the giraffe (1952), in the marmoset (1954) and in the wolf (1955). These relations were interpreted by HANSTRÖM to mean that the secretion is transported in the axoplasm in the form of a "Gomori-negative" substance which is transformed into a "Gomori-positive" one at the border of the axolemma. The proximodistal flow of plasm is a well-established fact (WEISS and HISCOE 1948, lit. also in SCHARRER and SCHARRER 1954). It may be possible that secretory material in the form which could not be registered by the Gomori method is continuously transported from the cell bodies by this perpetual flow. If this be the case the secretory particles are "Gomori-negative" to the extent that they occur at a submicroscopical level in the axoplasm and it is first when these accumulate to form aggregates measuring more than 200 mμ that they will be seen in the light microscope as the granules in Figs. 5 and 6. This interpretation, however, does not exclude the existence of a genuine "Gomori-negative" form of the secretion.

Besides the present observations the electron microscopical studies on the diencephalic neurosecretory system seem to support such a point of view. Granules considered to be neurosecretory have been described by several authors and the following sizes have been measured, PALAY (1955, 1957) 100—150 mμ, FUJITA (1957) 100—300 mμ, BARGMANN and KNOOP (1957) 120—180 mμ and DUNCAN (1958) 100—200 mμ (lit. in BARGMANN 1958). This confirms that a usual form of neurosecretory units has sizes below the resolving power of the light microscope (200 mμ), a fact which has also been emphasized by BARGMANN (1958).

Undoubtedly the formation of larger aggregates occurs in the cell bodies as finer granules or droplets. When these have left the cell body the proximal part of the axon is filled with droplets as shown in Fig. 7. The commonest form of secretion in proximal regions are the discussed perpherical substances in combination with larger droplets. The latter might be formed by an accumulatory effect and fusion of minute particles (Fig. 6). The constant phenomena described showing that the droplets only occur in the proximal parts of the tracts may be due to changes in the viscosity of the secretion constituting these droplets. These

are transformed into a state which is accumulated and homogenously dispersed in the distal parts of the axons and which gives the completely positive reaction to chrome haematoxylin.

SCHARRER and SCHARRER (1954) note in their abstracts of neurosecretion the great variations of the secretory process which may show a typical character for each species. It has been shown in several species that the secretory material completely fills the cell bodies before it is delivered to the axons and that the secretory process runs through a cycle whose last step is the total emptying of the secretion from the cell body. However in the roach the present observations may support an interpretation that the secretion is continuously delivered to the axons partly as granules and droplets but also in units at a submicroscopical level. The latter form of secretion is in the tracts accumulated to form larger particles and droplets which consequently do not always issue from the cell bodies as such large droplets. The fact must not be disregarded that there occurs an accumulation of secretory granules in the cell bodies which may have a storage capacity and that a cycle may take place in conformity to the cycles discussed by SCHARRER and SCHARRER (1954).

To sum up, the morphology of the secretory process in nucleus praeopticus shows considerable variations. The secretory material may appear as droplets and granules in the cell bodies but the presence and departure of secretory units below the resolving power of the light microscope from the cell bodies seem to be facts which must be taken into consideration in the study of the histogenesis of the praeoptico-hypophysial system of the roach.

The histogenesis of the praeoptico-hypophysial system in the roach

Investigations proved that no critical stage of actual interest in this investigation was reached before hatching. Until 13—14 days (11°C) after fertilization when the eggs were just about to hatch no morphological indications of a secretion-producing praeoptico-hypophysial system exist. Consequently the studies were concentrated to the stages after hatching.

At hatching-time the yolk-filled belly sack is a dominating feature of the larva and the newly hatched spawn attach themselves to the walls or the vegetation of the aquarium by means of a larval head gland. During the inactive period of attachment which lasts for about 30—36 hours (room temperature) most of the yolk is consumed and only a few yolk granules remain when the nektonic life starts.

Neurohypophysis. The "attachment-stage" 5.9—6.1 mm. The larva has a pituitary in the form of a rounded body and substances which take chrome haematoxy-

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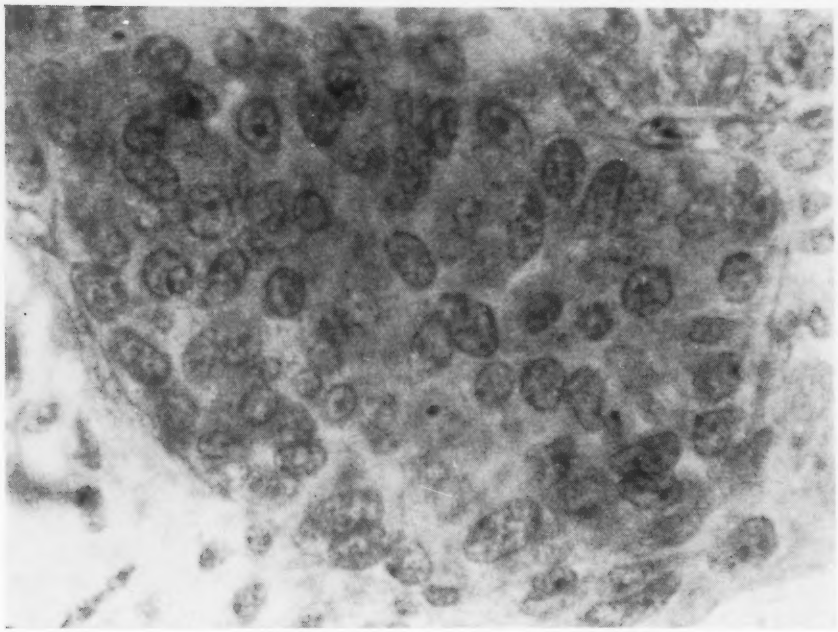

Fig. 8. The 6 mm stage. The pituitary of a newly hatched spawn before the free-swimming life starts. No vessels have yet invaded the organ. (Sag. 4 μ, Cr.-Htx., 1 500 ×)

lin or Astrablau are not present and vessels have not yet invaded the organ (Fig. 8).

In the brain only two areas exhibit a secretory activity which is demonstrated by the methods used. The actual areas are the flexural organ at the plica ventralis encephali in mesencephalon and the subcommissural organ (Olsson 1956). The conditions are in agreement with the observations of Olsson and his interpretation of the origin of Reissner's fibre. When the nektonic life begins the flexural cells have ceased to be secretionally active and the fibre issues exclusively from the subcommissural organ.

6.2 mm, two days after hatching, the first nektonic stage. Towards the end of the "attachment-stage" the pituitary shows considerable morphological changes. The rounded shape is abandoned and the pituitary has become flattened out to a rather thin plate in immediate connection with hypothalamus but has no stalk differentiated. In the dorsal part infundibulum appears with few ventrally migrating glioblasts and in this region the vessels are seen. Infundibulum and the developing neurohypophysis are characterized by the presence of small granules which are stained by chrome haematoxylin and Astrablau. They are present in all series of this stage and are at the limit at which they can be photographically established (Fig. 9).

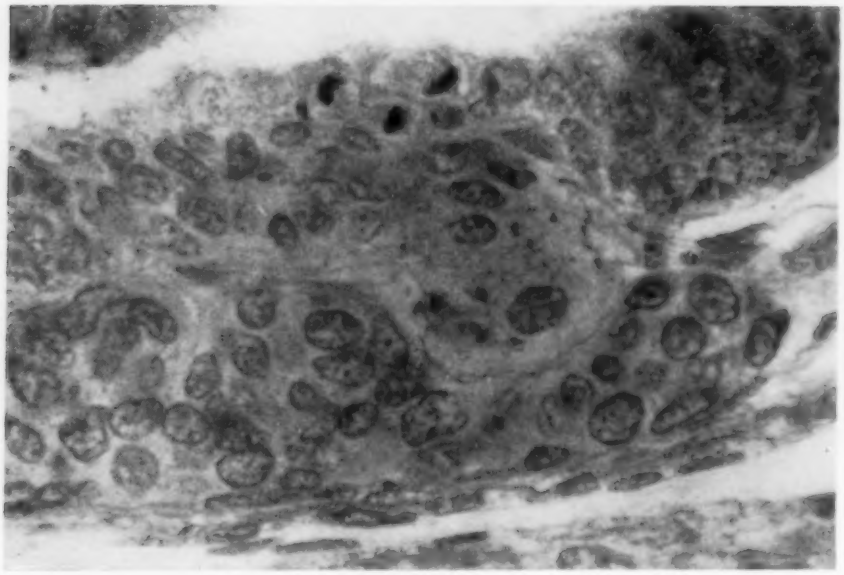

Fig. 9. The 6.2 mm stage. The pituitary of a spawn which has just begun the nektonic life. Note the vessels and the sparse granules within the nervous part of the pituitary. (Sag. 4 μ, Cr.-Htx., 1 500 ×)

6.3 mm, three days after hatching. The larva has quite resorbed the yolk and has been nektonic for about 24 hours. The nervous part of the pituitary has expanded considerably and undoubtedly the material which reacts positively with Astrablau has increased in quantity.

6.5—6.6 mm, four days after hatching. The pituitary has been invaded by vessels in its nervous part and around the vessels chrome haematoxylin stained substances are visible (Fig. 10). The glial cells in this part show no intimate relationship to these substances.

6.9—12 mm. These stages show a successive increase of the secretory matter in the neurohypophysis. First at the 11 mm stage (22 days after hatching) can the secretory material be traced in the axons in a proximal direction as far as to the region where tractus praeoptico-hypophyseus enters into the infundibulum.

19—70 mm. The pituitary is at the 19 mm stage differentiated in its main parts and has a well-developed stalk. The neurohypophysis is built up as in the adult of axons diverging into the adenohypophysis. The axons show variations in staining intensities. Some have just a blue tone of colour while others are dark blue often in combination with a beaded appearance. Round the vessels the secretion is rich and the axons can on account of their secretory content be traced to the proximal part of infundibulum.

The further development is characterized by an increase of the secretion in the

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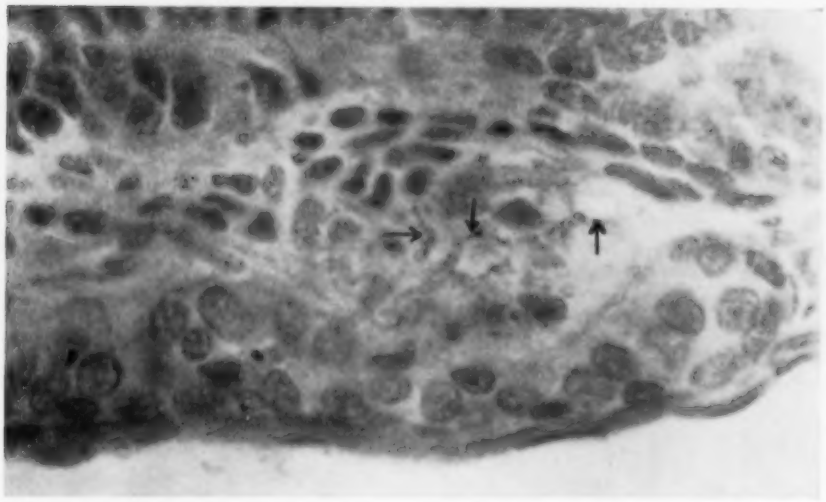

Fig. 10. The 6.6 mm stage. The caudal part of the pituitary with vessel surrounded by neurosecretory granules (arrows). (Sag. 4 μ, Cr.-Htx., 1 500 ×)

neurohypophysis. At the 35 mm stage the secretory matter occurs more conspicuously in infundibulum in the shape of beads which in size come close to those defined as Herring bodies. Remarkable however is the fact that the axons could not be traced further in a proximal direction than to the ventralmost part of the tuber region because of their lack of detectable secretion (Figs. 11 and 12).

In the 58 mm stage and later the axons within the neurohypophysis are homogenously stained all over and the conditions are equivalent to those of the adult.

Nucleus praeopticus. When the larva begins the free-swimming life and the first granules stained by chrome haematoxylin and Astrablau appear in the pituitary no morphological differentiation of nucleus praeopticus has started. Four days after hatching (6.5—6.6 mm) when secretion undoubtedly occurs in the pituitary the cells seem still as juvenile as in the preceding stages, and it is almost impossible to distinguish the thin border of protoplasm around the nucleus (Fig. 13). No secretion could be found. The cells are densely packed and the sizes of the cells in the future magnocellular part are equivalent to those of other cells in the praeoptic region. On the other hand several brain nuclei already at hatching show a considerable differentiation with larger neurons easy to determine by their Nissl substance and large nucleoli.

The development to 12 mm (22 days after hatching) involves small changes. The amount of cytoplasm has slightly increased and the nucleoli are more distinct but no typical features as peripherical Nissl substance appear. Further it is impos-

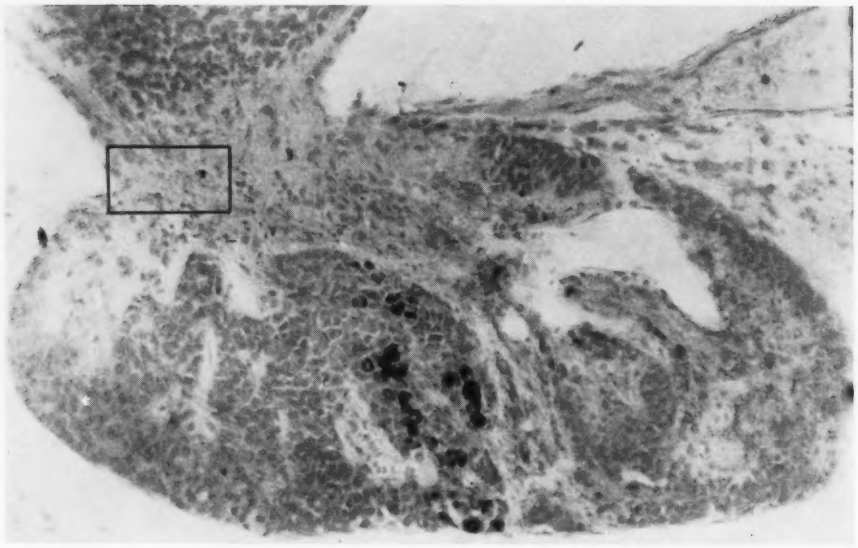

Fig. 11. The pituitary of the 35 mm stage (Sag. 5 μ, Cr.-Htx., 320 ×)

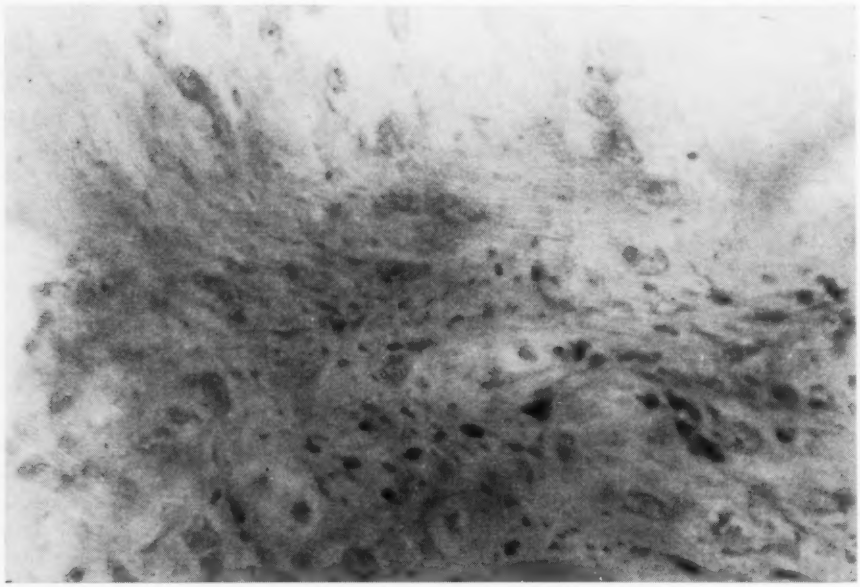

Fig. 12. Part of infundibulum (the outlined rectangle in Fig. 11, next section) showing the secretion in beads of the axons. The secretion could not be traced in amore proximal direction. (Sag. 5 μ, Cr.-Htx., 1 500 ×)

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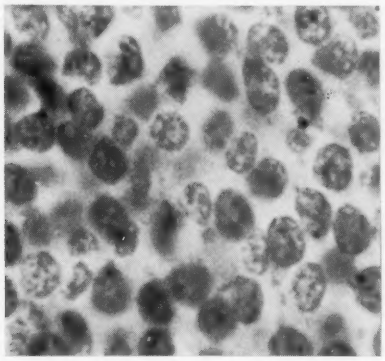

Fig. 13. Praeoptic cells of the 6.6 mm stage. (Sag. 4 μ, Cr.-Htx., 1 500 ×)

sible to establish morphological differences in the different parts of the praeoptic nucleus and no secretion can be detected.

19, 20 and 22 mm, 45 days after hatching. Nucleus praeopticus begins at these stages to assume the trapezium shape characteristic of the adult. The cells averaging 6 μ have now a clearly conspicuous cytoplasm. However in the dorsal part of nucleus praeopticus some cells reach sizes of 10—12 μ in diameter but still no characters as secretion, polymorphous nuclei or peripherical Nissl substance appear.

25, 29, 35, 37 and 40 mm, 100 days after hatching. A considerable variation of the sizes in the population is now a fact. In spite of the differences in sizes between 25 and 40 mm no important features distinguish these animals as far as the praeoptic nucleus is concerned. The magnocellular cells have more basiphil cytoplasm (Fig. 14). No secretion stainable with chrome haematoxylin or Astrablau is visible in the cell bodies.

58, 60, 68 and 78 mm. It was first at the 58 mm stage that secretion could be observed in a few cells of nucleus praeopticus and the specimens were more than a year old. The larger cells in pars dorsalis measure 18 μ and the smaller ones 12 μ. In the parvocellular part dorsal to recessus praeopticus the cells are about 9 μ in diameter. The magnocellular cells have a stronger basiphil reaction with a peripherical Nissl substance and some cells have traces of incipient nuclei-invaginations. However only a few cells show secretory matter as a fine granulation (Figs. 15 and 16). With the Astrablau method it was impossible to determine the secretion in other forms than in these granules.

Tractus praeoptico-hypophyseus. At the 11 mm stage the secretion could be found in tractus praeoptico-hypophyseus from infundibulum and further in a distal direction. This is a condition common to all stages investigated up to 58 mm. As a matter of fact it is first when the secretory material can be recognized in nucleus praeopticus that the tracts have secretion microscopically determinable

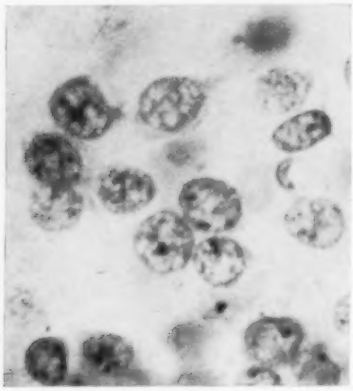

Fig. 14. Praeoptic cells of the 35 mm stage. (Sag. 4, μ, Cr.-Htx., 1 400 ×)

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in all their length and consequently can be traced on Gomori slides. It seems clear however that by no means all axons in the tracts have noticeable secretion in their proximal parts. Instead only a few fibres can be followed from nucleus praeopticus but in the tuber region the axons with secretion have increased in number and are as in the adult homogenously filled with secretion.

Discussion. The histogenesis of the praeoptico-hypophysial system of the roach is characterized by the appearance of the secretory matter in the pituitary when the nektonic life starts but it is not until the 58 mm stage that secretion can be clearly detected in a few cell bodies. Of all the specimens investigated these conditions are the most extreme example of the late appearance of secretion in the praeoptic nuclei and are similar to MAZZI's result (1954) according to which the neurohypophysis in Rana agilis has secretion at 11.3 mm but the praeoptic nucleus not until 37.5 mm. In most studies of the histogenesis of the diencephalic neurosecretory system the species independent of class often only differ in time between the appearance of the secretion in the pituitary and the neurosecretory cells. This has caused different and controversial opinions on the secretory process. According to the schools of Scharrer and Bargmann the secretory process is located to the cell body and the secretion transported by the continuous flow of axoplasm to be stored and released within the pituitary. As far as the microscopical studies on the histogenesis of the systems are concerned this theory does not seem to be confirmed by the first detectable secretion in the neurohypophysis. This has been considered by DIEPEN et al. (1954) to be a strong indicium against this transport theory. According to the same authors the poorness of secretion in the neurosecretory cells of the adult in several species but the constant great amounts in the neurohypophysis does not favour this theory (cf. DIEPEN 1954). Instead the terminal regions of the neurosecretory system are considered to be the most important region for the synthesis of secretion, an opinion also expressed

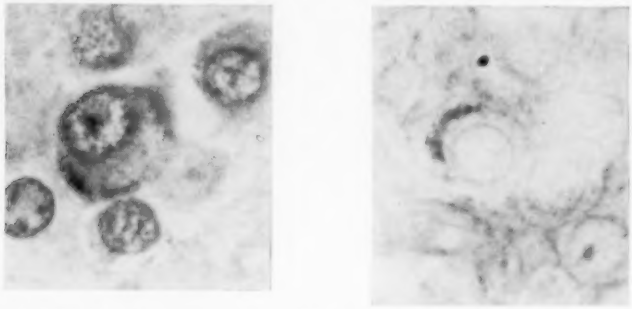

Fig. 15 and 16. The first detectable secretion in the praeoptic nuclei appears as a fine granulation in the cell body. Fig. 15. The 58 mm stage. (Sag. 4 μ, Cr.-Htx., 1400 ×) Fig. 16. The 68 mm stage. (Sag. 4 μ, Astrablau 1400 ×)

by Bodian (1951). This view based on light microscopical studies can only be valid on the assumption that the secretory matter always occurs in forms over the resolving power of the light microscope. The basis for controversial interpretations of the origin of the secretion seems however not to exist when Diepen et al. do not deny the importance of the cell body and the nucleus in the synthesis of the secretion. Further the presence of a genuine "Gomori-negative" precursor form of the secretion cannot be excluded. Wingstrand (1953) found that the secretion appears simultaneously in the proximal and distal parts of the system in the chick and extracts from the hypophysial region of chick embryos had an antidiuretic effect three days before the secretion could be established on Gomori slides. One of the possible explanations given was that the neurosecretory substances may occur in such small amounts that they could not be demonstrated with the chrome haematoxylin method. This point of view has also been expressed by Mazzi (1954), Scharrer and Scharrer (1954) and Rodeck and Caesar (1956) who considered the possibility of the submicroscopical departure of secretion from the cell bodies and consequently that it is not seen until sufficient amounts have accumulated in the pituitary. Not until a certain concentration of secretion has been reached does the chrome haematoxylin method give a positive reaction.

In the adult roach it has been demonstrated that many indicia are present for assuming that secretory material is synthesized in the cell bodies in forms which are not detectable by the Gomori method (1941) and that the secretion may depart from the cell bodies at a submicroscopical level. Only when the secretion has accumulated in the tracts and in the neurohypophysis is it noticeable. Because of that it is not surprising that the ontogenesis is characterized by the first appearance of secretion within the neurohypophysis. As a matter of fact the occurrence of the secretion in a submicroscopical form seems to be the natural condition when the juvenile system has just started the production of secretion. Not until the roach is about 58 mm in length has the secretion production reached such a

quantitative level that the secretory matter is detectable in some cell bodies in nucleus praeopticus. However this is an enigmatic contrast to the results of ARVY et al. (1956) who found the first visible secretion in nucleus praeopticus of the salmon three days after hatching. Variations between the species in the morphology of the secretory process with characters specific to each species (SCHARRER and SCHARRER 1954) may exist already in the early stages. The secretion in nucleus praeopticus of the salmon is accumulated in the cell bodies to form visible aggregates soon after hatching which does not happen in the roach until later stages in the histogenesis.

Summary

The secretory process in nucleus praeopticus of the adult roach (L e u c i s c u s r u t i l u s) and the histogenesis of the praeoptico-hypophysial system in the same species were studied. In several adult specimens the secretion in the praeoptic cells is dispersed in the cytoplasm only demonstrable by the histochemical methods. The occurrence of the secretion in a minute concentration and in submicroscopical units in the basiphil cytoplasm was assumed to be the explanation of the negative reaction to the Gomori method. Indicia point to the departure of the secretion from the cell bodies in this form not detectable with the chrome haematoxylin.

During the ontogenesis the first neurosecretory substance appears in the pituitary when the nektonic life starts (6.1 mm) but not until a year later (50—58 mm) could the secretion be established in a few cells of nucleus praeopticus and only at these stages could the tracts be followed in all their length by their secretory content. The studies on the adult in combination with these observations indicate that the juvenile system may be characterized by a secretion at a submicroscopical level and that the existence of these substances cannot be proved in the light microscope until accumulated in sufficient amounts in the distal parts of the system.

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